# DAIRY CHEMISTRY AND PHYSICS

# Dairy Chemistry and Physics

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*This book is dedicated to Hendrik Mulder, formerly Professor of Dairying at the Agricultural University of Wageningen, and to the memory of Leroy Sheldon Palmer, formerly Professor of Agricultural Biochemistry at the University of Minnesota. These men, our academic mentors, were both outstanding pioneers in teaching and research in dairy chemistry and physics.*



[illegible]

# Preface

Milk and products made from it affect the lives of a large proportion of the world's population. Many dairy products are consumed at times and places far removed from the point at which the milk was produced. This is made possible by the chemical and physical treatments and fractionations applied to milk by modern technology. These treatments are designed to preserve the nutritional value of the milk constituents in the form of palatable products. As food technology in general becomes more advanced and more sophisticated, there is less need for specific commodity technology; on the other hand, there is more need for specific knowledge of raw materials and the effects of various processing treatments on them.

In this book we attempt to present a reasonably complete and integrated picture of the chemistry and physics of milk. The book is intended as a text and general reference for students and others who are interested in the various aspects of production, processing, inspection, and nutritive value of milk. It deals almost exclusively, except for comparison of the nutritive values of bovine and human milks, with milk of domestic cows. Our aim is to describe the milk system in terms of composition and physical structure, properties of the constituents, and chemical and physical changes that occur during storing and processing. We deal with the principles of dairy processes but do not discuss products such as butter and cheese, which differ greatly from milk; separate volumes would be required for each.

The discussion of chemical changes includes some biochemical processes; biosynthesis and secretion of milk are dealt with in an introductory chapter as a background for understanding variations in composition and structure. Some aspects of the book should be valuable to microbiologists, although microbiological problems are not treated systematically or exhaustively. We consider processes and structures not involving transfer of electrons as

"physical." Thus, we include phenomena that might be designated "physicochemical" in this category. In particular, colloidal phenomena are described in more detail and with greater rigor than in any previous treatment of the subject. No doubt, many readers will find this section more difficult than the others. We think that this situation arises, in part, because past courses in food science have emphasized chemical more than physical principles and, in part, because we, at least, know of no way to simplify the treatment without sacrificing rigor.

One chapter is a summary and critique of methods for analysis of the composition of milk, but we have not dealt to any extent with specialized analytical methods that might be used as research tools in the study of milk and its constituents.

We present the book in the style and format of a text, not as a monographic review of literature. Thus, direct references, citations of literature, and tabulations of actual detailed data are omitted. Suggested literature for supplementary reading is given at the end of each chapter; tables and figures are constructed on the basis of our interpretation and integration of available data; and a large amount of factual data are presented in the appendices.

The literature of milk chemistry and physics is vast; indeed, in some aspects it is nearly overwhelming. Many facets of the subject are, however, still controversial. We feel that elimination of controversial issues would unduly restrict the coverage we wish to attain. In such cases we have not attempted to present all points of view but have selected those that seem in best accord with experimental data and basic science and have expressed them with qualifying phraseology.

The level of treatment demands a knowledge of mathematics through simple calculus, and introductory organic, physical, and biological chemistry. For the benefit of those who may not be familiar with the physical chemistry of colloids and surfaces, a chapter on basic aspects of the subject is included.

We gratefully acknowledge the help and encouragement given us by the following people, who have read and criticized drafts of one or more of the chapters or sections: Dr. H. T. Badings (Ede), Dr. V. A. Bloomfield (St. Paul), Dr. T. J. Buma (Groningen), Dr. D. T. Davies (Ayr), Dr. H. van Duin (Ede), Dr. P. F. Fox (Cork), Dr. J. E. Gander (St. Paul), Dr. L. M. Henderson (St. Paul), Dr. J. Hiddink (Ede), M. C. van der Haven (Wageningen), Dr. C. Holt (Ayr), Dr. M. Karel (Cambridge, Mass.), Dr. J. Koeman (Wageningen), Dr. J. Koops (Ede), Dr. B. L. Larson (Urbana), Dr. W. Manson (Ayr), Dr. G. K. Murthy (Cincinnati), Dr. A. Noomen (Wageningen), Dr. C. J. Olling (Leeuwarden), Dr. S. Patton (San Diego), Dr. T. A. J. Payens (Ede), Dr. A. Prins (Wageningen), Dr. T. Richardson (Madison), Dr. D. G. Schmidt (Ede), Dr. J. W. Sherbon (Ithaca), Dr. J. J. Stadhouders (Ede), R. A. Visser (Leeuwarden), Dr. T. van Vliet (Wageningen), Professor E. A. Vos (Wageningen), H. Werner (Hillerød), and Dr. J. N. de Wit (Ede).

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*September 1983*

# CONTENTS


List of Units and Symbols xvii

**1. Outline of Milk Composition and Structure** 1

1.1. Principal Components 1
1.2. Structure 3
1.3. Some Properties 8
1.4. Variability 8
1.5. Processing 10
Suggested Literature 11

**2. Biosynthesis and Secretion of Milk** 12

2.1. The Mammary Gland 12
2.2. Precursors of Milk Constituents 14
2.3. Biosynthetic Processes 17
2.4. Transport Processes 21
2.5. Cells and Cell Fragments in Milk 24
2.6. Limitations on Milk Composition 24
2.7. Changes in Milk in Ducts and Cisterns 25
Suggested Literature 26

**3. Carbohydrates** 27

3.1. Chemical Properties of Lactose 27
3.2. Physicochemical Aspects of Lactose 30
3.3. Fermentation 37
3.4. Other Carbohydrates 40
Suggested Literature 40

**4. Salts** 42
4.1. Overall Composition 42
4.2. The Salt Solution: Composition 44
4.3. The Salt Solution: Properties 49
4.4. The Colloidal Calcium Phosphate 51
4.5. Changes in Salts 53
Suggested Literature 57

**5. Lipids** 58
5.1. Composition 58
5.2. Properties of Various Lipids 66
5.3. The Fat Globule Membrane 70
5.4. The Fat in Milk Products; Modification 76
5.5. Lipolysis 78
5.6. Autoxidation 83
5.7. Crystallization 88
Suggested Literature 96

**6. Proteins** 98
6.1. Chemistry and Conformation of Proteins 98
6.2. Protein Composition of Milk 106
6.3. Caseins 109
6.4. Whey Proteins 114
6.5. Fat Globule Membrane Proteins 121
Suggested Literature 122

**7. Enzymes** 123
7.1. Kinetics of Enzyme-Catalyzed Reactions 123
7.2. Source and Significance of Enzymes in Milk 126
7.3. Some Prominent Milk Enzymes 126
7.4. Inactivation 133
Suggested Literature 134

**8. Miscellaneous Components** 135
8.1. Natural Components 135
8.2. Contaminants 140
8.3. Radionuclides 143
Suggested Literature 145

**9. Variability** 146
9.1. Sources and Nature of Variation 146
9.2. Extent of Variation 155

9.3. Correlations Among Compositional Parameters 157
9.4. Some Consequences 159
Suggested Literature 161

**10. Heating** **162**
10.1. Overview of Changes 162
10.2. Kinetics 167
10.3. Reactions of Proteins 173
10.4. Browning Reactions 177
10.5. Fouling 181
Suggested Literature 184

**11. Some Properties** **186**
11.1. Density 186
11.2. Optical Properties 188
11.3. Acidity 192
11.4. Redox Potentials 197
11.5. Redox Reactions and Photooxidation 201
11.6. Milk as a Substrate for Bacteria 206
Suggested Literature 210

**12. Colloidal and Surface Phenomena** **211**
12.1. Surface Chemistry 211
12.2. Colloidal Stability 218
12.3. Changes in Dispersity 224
12.4. Size Distributions 226
Suggested Literature 227

**13. Casein Micelles** **229**
13.1. Properties 229
13.2. Colloidal Stability 234
13.3. Renneting 237
13.4. Age Gelation 246
13.5. Heat Stability 248
Suggested Literature 253

**14. Milk Fat Globules** **254**
14.1. Properties 254
14.2. Emulsion Stability 258
14.3. Cold Agglutination 264
14.4. Homogenization 266
14.5. Creaming 273
Suggested Literature 278

**15. Interactions of Milk with Air Bubbles** 279
15.1. General Aspects of Foaming 279
15.2. Foaming of Milk Products 282
15.3. Churning and Whipping 283
15.4. Ice Cream 287
Suggested Literature 289

**16. Rheological Properties** 290
16.1. Newtonian Behavior 290
16.2. Non-Newtonian Liquids 292
16.3. Gels 294
16.4. Milk Fat 298
Suggested Literature 299

**17. Properties of Milk Concentrates** 301
17.1. Water Relations 301
17.2. Effects of Concentrating 307
17.3. Evaporation 314
17.4. Freezing 315
17.5. Drying 317
17.6. Membrane Processes 320
17.7. Protein Preparations 323
17.8. Reconstitution and Recombination 331
Suggested Literature 334

**18. Flavors and Off-Flavors** 336
18.1. General Aspects 336
18.2. The Flavor of Milk 342
18.3. Off-Flavors in Milk and Dairy Products 343
18.4. Flavor of Milk Products 350
Suggested Literature 357

**19. Nutritive Value** 358
19.1. General Aspects 358
19.2. Carbohydrates 359
19.3. Minerals 362
19.4. Lipids 363
19.5. Proteins 365
19.6. Vitamins 368
19.7. Milk Products 373
Suggested Literature 375

**20. Analysis of Composition** 377
20.1. General Aspects 377

20.2. Solids and Solids-Not-Fat 381
20.3. Fat Content 382
20.4. Proteins 384
20.5. Carbohydrates 388
20.6. Salt Constituents 391
Suggested Literature 393

**Appendix** **395**

**Index** **459**

# List of Units and Symbols

For the most part numerical data given in this text are expressed in the International System (SI) of Units. Basic units in this system are:

| Quantity | Name | Symbol |
|---|---|---|
| Length | meter | m |
| Mass | kilogram | kg |
| Time | second | s |
| Electric current | ampere | A |
| Temperature | kelvin | K |
| Amount of substance | mole | mol |

Important derived units used herein are as follows:

| Quantity | Name | Symbol | Expression in Basic Units |
|---|---|---|---|
| Force | newton | N | $kg \cdot m \cdot s^{-2}$ |
| Pressure | pascal | Pa | $kg \cdot m^{-1} \cdot s^{-2}$ |
| Energy | joule | J | $kg \cdot m^2 \cdot s^{-2}$ |
| Electromotive force | volt | V | $kg \cdot m^2 \cdot A^{-1} \cdot m^{-3}$ |
| Electric conductivity | | $A \cdot v^{-1} \cdot m^{-1}$ | $A^2 \cdot s^3 \cdot kg^{-1} \cdot m^{-3}$ |

The recommended prefix symbols M, k, m, $\mu$ and n are used respectively for powers of $10^6$, $10^3$, $10^{-3}$, $10^{-6}$ and $10^{-9}$ of the units.

Some lengths are given in Ångstroms ($10^{-10}$ m); symbol Å. Temperature levels are given in degrees Celsius (°C) and masses often in grams ($10^{-3}$ kg). Volumes are in liters ($10^{-3}$ m$^3$) in a few cases. Concentrations are usually in mass units per kilogram, but for some purposes molar concentrations (mol·liter$^{-1}$ = kmol·m$^{-3}$) are used. The latter are called molarity and symbolized *M*.

## Some Conversions of SI Units

Mass density: 1 kg·m$^{-3}$ = $10^{-3}$ g/ml
Pressure or stress: 1 MPa = $10^6$ N·m$^{-2}$ = 10 bar (atm) = 145 psi
Surface tension: 1 mN·m$^{-1}$ = 1 dyne/cm
Energy: 1 J = 0.239 cal = $10^7$ erg
Viscosity: 1 mPa·s = 0.01 Poise = 1 cP

## Frequently Used Symbols for Properties

| | |
|---|---|
| A | Area |
| $a$ | thermodynamic activity; acceleration |
| $a_w$ | water activity |
| $c$ | concentration; constant |
| $d$ | diameter |
| $d_{vs}$ | volume—surface average diameter |
| $n$ | refractive index |
| $p$ | pressure |
| $t$ | time |
| $T$ | temperature (in K if unspecified) |
| $\gamma$ | surface or interfacial tension |
| $\Gamma$ | surface excess (quantity adsorbed per unit area) |
| $\eta$ | viscosity |
| $\lambda$ | wavelength |
| $\rho$ | mass density |
| $\sigma$ | standard deviation |
| $\phi$ | volume fraction |

# DAIRY CHEMISTRY AND PHYSICS

# 1

# Outline of Milk Composition and Structure

Milk is a complex fluid containing many components in several states of dispersion. Understanding its properties and the many changes that can occur in it requires knowledge of all of the components and their effects on one another. This chapter presents a general overview of the components of milk and their physical states of dispersion, introduces some terms and definitions, and points out pitfalls that are sometimes encountered. The chapter is intended to make the rest of the book easier to understand; most of the subjects are discussed in greater detail in other chapters.

Milk is the secretion of the mammary glands of mammals and is intended for nutrition of the young. In this book, unless otherwise stated, the word *milk* is used for the mammary secretion, excluding colostrum, of healthy cows (*Bos taurus* and *Bos indicus*).

## 1.1 PRINCIPAL COMPONENTS

The composition of milk determines its nutritive quality, its value as a raw material for making food products, and many of its properties. A rough classification of the principal constituents of milk with approximate percentages of contents is given in Table 1.1. The principal components are those present in the largest concentrations; they are not necessarily the most important ones in all respects. For instance, the vitamins, which are present

**Table 1.1. Approximate Composition of Milk[a]**

| Component | Average Content Percentage (w/w) | Range[b] Percentage (w/w) | Average Percentage of Dry Matter |
|---|---|---|---|
| Water | 87.3 | 85.5–88.7 | |
| Solids–not–fat | 8.8 | 7.9–10.0 | 69. |
| Fat in dry matter | 31. | 21.–38. | |
| Lactose | 4.6 | 3.8–5.3 | 36. |
| Fat | 3.9 | 2.4–5.5 | 31. |
| Protein | 3.25 | 2.3–4.4 | 26. |
| casein | 2.6 | 1.7–3.5 | 20. |
| Mineral substances | 0.65 | 0.53–0.80 | 5.1 |
| Organic acids | 0.18 | 0.13–0.22 | 1.4 |
| Miscellaneous | 0.14 | | 1.1 |

[a]Data for Netherlands milk but typical of milks of lowland breeds, such as Friesians, Red Danes, Holsteins, Ayrshires.
[b]These values will be exceeded rarely; for example, in 1–2% of samples of separate milkings of individual cows, excluding colostrum and milk drawn shortly before parturition.

in low concentrations, are important in nutritive value; enzymes are important catalysts of deteriorative reactions; and some minor components contribute markedly to the taste of milk. More information on the major components is in Table 1.3, in the respective chapters on components, and in the appendix.

Lactose is the distinctive sugar of milk; other carbohydrates are present only in traces. Lactose is a reducing disaccharide composed of glucose and galactose. It gives milk a slightly sweet taste. It is the principal carbon source for most of the microorganisms that grow in milk.

Milk fat consists of numerous different lipids. More than 98% of milk fat is made up of triglycerides. Other lipids that are present include cholesterol, diglycerides, free fatty acids, phospholipids, and cerebrosides. The component fatty acids of milk lipids exhibit a remarkably wide range of 4–20 carbon atoms and 0–4 double bonds.

There are several kinds of milk proteins often classified as caseins, which are insoluble at pH 4–5, and whey or serum proteins. Caseins represent about 80% of the total milk proteins. Milk contains numerous "minor proteins," notably enzymes, which are negligible in weight but not in action. Sometimes "protein content" is calculated merely by multiplying total nitrogen by a certain factor. However, it should be remembered that about 5% of the nitrogen of milk is present in the form of small molecules and is called nonprotein nitrogen.

The minerals in milk are mainly inorganic salts, partly ionized and partly present as complex salts. Some inorganic matter is bound covalently, such as phosphate groups in caseins. Often, milk is ashed to determine "salt content." Ash does not represent milk salts truly because organic salts are destroyed by ashing and some nonsalt components (e.g., sulfur of amino acids) contribute to ash. Milk contains numerous other elements in trace quantities. Salts of organic acids occur in fresh milk; citrate is the principal one.

Milk has many miscellaneous components. For example, all vitamins are present. As analytical techniques improve, more components are being identified.

Altogether, milk probably contains on the order of 100,000 different molecular species, but most of these have not been identified, let alone isolated; their existence can be inferred only from a reaction or other circumstantial evidence. Even for an identified substance, it is sometimes not known whether it is present as a separate component or as a building block of a larger molecule. Different methods of determination may give somewhat different results.

Sometimes, common terminology is not entirely clear. For instance, the "lysine content of milk" usually would mean the lysine residues of the protein rather than the minute quantity of free lysine present. The same holds true for numerous other substances. It is not correct to speak of "NaCl content" of milk because milk contains predominantly Na and Cl ions, either free or bound. "Phosphorus content" usually would mean P present as phosphate, either inorganic (free) or organic (bound by ester bonds to an organic constituent). Caution often is needed in interpreting the terminology used in reference to milk and its components.

## 1.2. STRUCTURE

The properties of a biological material are not determined fully by its composition, and milk is no exception. The physical structure (i.e., the arrangement of the components in space) must also be known. Furthermore, the interactive forces between components are important; they determine the integrity of the whole system under various conditions. This is discussed in Chapters 11–14. In this section, we will concentrate on structure.

Milk has a relatively simple and well-studied structure. The main structural elements are shown schematically in Figure 1.1. They are microscopic or submicroscopic in scale; we are not considering molecular structure at this point. Some properties of the structural elements of milk are given in Table 1.2 in a somewhat simplified form; the numerical data are meant only to define orders of magnitude.

Fat globules in milk are more complicated than simple emulsion droplets because of their membrane. This is unlike an adsorption layer; it is derived

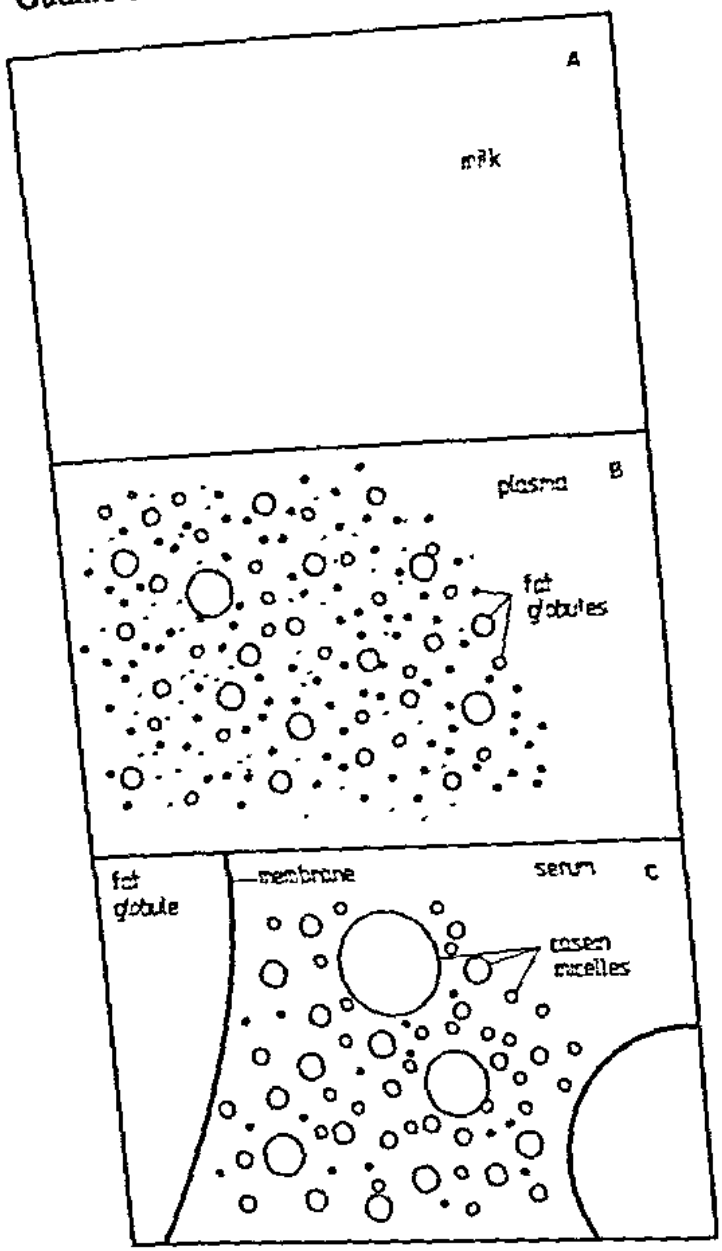



**figure 1.1.** Milk viewed at different magnifications. The picture indicates the relative size of structural elements. (A) Magnified × 5. Uniform liquid. However, the liquid is turbid and thus cannot be homogeneous. (B) Magnified × 500. Spherical droplets, consisting of fat. These globules float in a liquid—plasma—this is still turbid. (C) Magnified × 50,000. The plasma contains proteinaceous particles, casein micelles. The remaining serum is still opalescent (so it must contain other particles). The fat globules have a thin outer layer—membrane—of different constitution. From H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

from, but not identical to, the apical membrane of the mammary secretory cell. (See Section 2.4.3.) The mass of the membrane is about 2% of that of the total fat globules. It consists mainly of polar lipids and proteins, and it includes many enzymes. Milk fat and fat globules are not identical, because about half of the membrane is not lipid material and about 0.4% of the fat of milk is found outside the globules.

Milk minus fat globules is called *milk plasma* It is almost but not quite equal to separated milk (skim milk), separation is never complete In this book, *plasma* and *serum* will refer to physical fractions of milk—not blood—although these terms were coined in reference to blood plasma and blood serum

Casein micelles consist of water, casein, salts, and some minor compo-

**Table 1.2. Properties of the Main Structural Elements of Milk, Including Approximate Numerical Values**

| | | Plasma | | |
|---|---|---|---|---|
| | | | Serum | |
| | Fat Globules | Casein Micelles | Globular Proteins | Lipoprotein Particles |
| Main components | Lipids | Casein, water, salts | Serum proteins | Lipids, proteins |
| To be considered as | Emulsion | Fine dispersion | Colloidal solution | Colloidal dispersion |
| Content (% dry matter) | 3 8 | 2 8 | 0 6 | ~0 01 |
| Volume fraction | 0 042 | ~0 06 | 0 006 | ~0 0001 |
| Particle diameter[a] | 0 1–10 $\mu$m | 10–300 nm | 3–6 nm | ~10 nm |
| Number per ml | $10^{10}$ | $10^{14}$ | $10^{17}$ | $10^{14}$ |
| Surface area ($cm^2$/ml milk) | 700 | 40,000 | 50,000 | 100 |
| Density (~ 20°C) (g/ml) | 0 92 | 1 11 | 1 34 | 1 10 |
| Visible with | Microscope | Ultra microscope | (Electron microscope) | (Electron microscope) |
| Light scattering | Very turbid, white | Turbid, bluish | Light haze | Negligible |
| Separable with | Dairy separator | High speed centrifuge | Gel filtration | Gel filtration |
| Filterable with | Fine sintered glass | Chamberland filter | Cellophane | Cellophane |
| Diffusion rate (mm in 1 h) | — | 0 1–0 3 | 0 6 | 0 4 |
| Flocculation | By "agglutinin" in the cold | By acid or by rennet | By heat | |
| Isoelectric pH | ~3 8 | ~4 6 | 4–5 5 | ~4? |

[a] 1 mm = $10^3$ $\mu$m = $10^6$ nm = $10^7$ Å
*For comparison most molecules and ions in solution are 0 4–1 0 nm diameter and diffuse in the order of 5 nm in 1 h*

nents including lipase and proteinase. A trace of the casein is in solution, not in the micelles. Casein binds cations fairly strongly, notably Ca and Mg ions at the pH of milk (~ 6.7); thus, it is said to be present as a caseinate. Casein micelles contain amorphous calcium phosphate and a small amount of citrate. This often is called colloidal phosphate, not because it is present in a separate colloidal state but because it is part of colloidal particles. The whole is called calcium–caseinate calcium–phosphate complex. Actually, the name *micelle* for the aggregate may be a misnomer; it is not a micelle in the colloidal sense. Nevertheless, the term is used widely, and we will employ it in this book.

Casein micelles are built of smaller particles called subunits or submicelles. These are roughly sperical and on the order of 10 nm in diameter. Water—or, strictly speaking, milk serum—occupies the space between subunits. The micelles have an open structure and contain at least 1.5 g of water per gram of protein.

Milk serum is defined as milk plasma minus casein micelles. Removal of the micelles from skim milk by clotting with rennet yields the liquid called whey. It is not quite equivalent to milk serum, mainly because it contains some polypeptides cleaved from casein by rennet.

Whey proteins are those present in whey. Strictly speaking, the nonmicellar proteins of milk should be called serum proteins, but this term is not used much. The whey proteins are mainly globular proteins, present as such or as small oligomers. They bind some counterions (cations) and a little water.

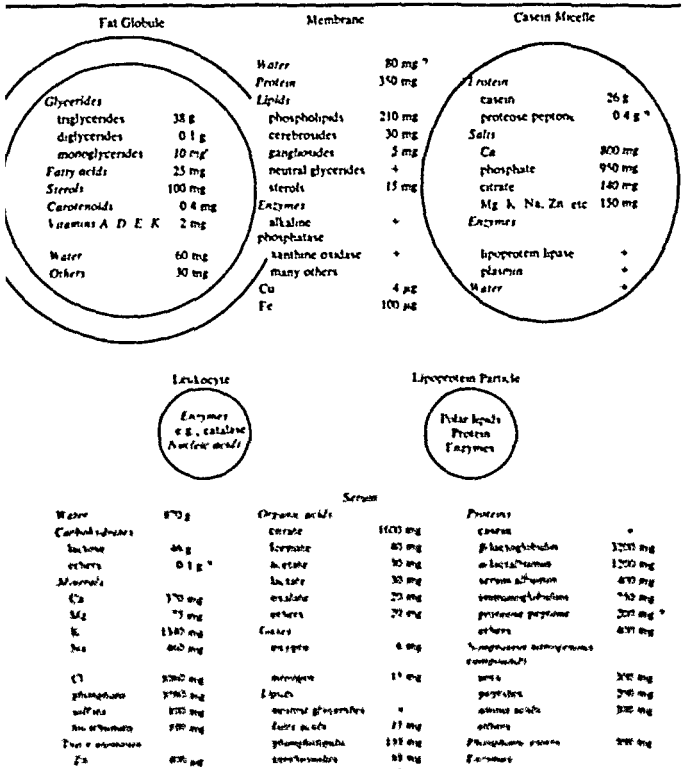
Lipoprotein particles, sometimes called milk microsomes, vary in quantity, composition, and shape. They consist of remnants of cell membranes, microvilli, and so on.

Somatic cells, mainly leukocytes of various types, may be considered extraneous particles, although they are always present. They are about 10 μm in diameter, number about 100,000 per milliliter, and account for about 0.005% of the volume of milk. They contain, of course, all cytoplasmic components, notably nucleic acids and enzymes. They are rich in catalase. In some milks, particularly those of mastitic cows, cell counts are much higher.

Table 1.3 gives a survey of milk structure and composition. Such data form the basis for calculating the composition of milk derivatives obtained by separation or other processing treatments. This must be done with caution, because several complications may arise:

1. Composition and structure may vary. (See Section 1.4.)
2. Separation may be incomplete.
3. Components, notably cations, may be partly adsorbed onto structural elements.
4. Not all water is available as a solvent.

## Table 1.3. Composition and Structure of Milk, Approximate Average Quantities in 1 kg Milk



## 1.3. SOME PROPERTIES

Milk contains more water than any other constituent, and the aqueous part constitutes the continuous phase. Thus, the properties of milk are primarily those of an aqueous system.

Polar substances dissolve well in milk. The low viscosity of milk (about twice that of water) enables it to be mixed easily, even by convection currents caused by relatively small temperature gradients. Because the structural elements are so small, diffusion into or out of them is achieved within a second. This implies a very rapid partition equilibrium between fat globules and plasma or between casein micelles and serum. It also means that apolar substances, especially volatiles, can be taken up by milk easily, because they are soluble in the fat globules; this is especially important for the uptake of odorous components from the air.

The substances dissolved in water give milk its osmotic pressure (~ 700 kPa), freezing point depression (~ 0.54 K), electrical conductivity (~ 0.5 $A \cdot V^{-1} \cdot m^{-1}$), pH (~ 6.7), ionic strength (~ 0.08 molar) and water activity (~ 0.993).

Milk can be modified easily. It has many components that can react, and many chemical changes take place particularly when milk is heated. Deliberate chemical modifications of milk seldom are used in dairy practice. Enzymatic transformations also are possible, and most microorganisms grow well in milk because of its wide range of nutrients and other favorable conditions (pH, $a_w$, redox potential). Hence, milk can be fermented in various ways. The phases can be separated by processes like centrifugation to yield skim milk and cream, or curdling to yield whey and curd. Moreover, the molecular separation processes common in the food industry, such as concentration, drying, membrane processes, and crystallization, can be applied. With these and some physical transformation processes, such as churning, a wide variety of products can be made from milk.

Milk is highly nutritious; it evolved that way. It contains all of the nutrients required by humans and other animals, though not all of them in significant quantities. It has a fairly bland taste; it is slightly sweet from lactose and slightly salty from milk salts; some minor components contribute to its flavor.

## 1.4. VARIABILITY

Although milk is, in a qualitative sense, fairly constant in composition and properties, there is considerable quantitative variation. Chemical composition, size and stability of structural elements, and physical properties may all differ among lots of milk. This may be the result of natural variation or changes occurring after the milk has been drawn.

Natural variation may have the following causes

1. Genetic, between breeds of cow or between individuals
2. Physiological, particularly stage of lactation, and also age of cow, estrus, and gestation
3. Environment, particularly feed, and also climate and stress

These variables and their effects are treated further in Chapter 9 Table 1 1 gives some idea about the variability in gross composition The largest variations are those between cows, stage of lactation, and feed (in the case of milk fat) Generally, milk yield is more variable than composition.

Milk is handled mostly in large quantities, comprising the milkings of many cows Such pooling reduces the variations The principal differences among lots of pooled milk are those caused by season, which is usually correlated with stage of lactation, and those between regions, which may differ in predominant breed or feeding practice

Up to this point, "abnormal milk" has not been discussed Although the distinction is vague and arbitrary, "abnormality" concerns

1. *Colostrum* The first drawn secretion after parturition differs greatly from normal milk It has very high serum protein content, and there is often some blood present
2. *Milk from a Diseased Udder, or Mastitic Milk* Its composition may be a little nearer that of blood serum It contains many leukocytes, and there are several other aberrations
3. *Contaminants Entering the Milk during or after Milking* These may be almost anything, such as disinfectants, pesticides, metal ions, plasticizers, and dust Most important is contamination with microorganisms The milk inside a healthy udder is sterile Proper milking and handling largely eliminate contamination, but some microorganisms inevitably gain entrance

It should be noted that extraneous components also may enter milk via the cow, generally through the feed Some extraneous contaminants (e g , pesticides) may be metabolized in passage through the cow Generally, the cow acts as a filter for extraneous substances, but the effectiveness of filtration differs greatly with the type of component

Large variations may occur because the milk changes after it is drawn Milk is not a system in equilibrium, thus, it may change even if it remains in exactly the same condition as in the udder This makes it difficult to distinguish between natural and other components Conditions also change contamination takes place, milk becomes exposed to air and light, the temperature usually is lowered Changes may be classified as follows

1. *Physical changes* include creaming of fat globules and crystallization of part of the fat on cooling. Fat globules may cream rapidly because they aggregate to loose, large flocs at low temperature (the so-called "cold agglutination"). Air bubbles enter the milk and form a new structural element. They may damage the fat globules, particularly if the fat is partially crystalline. Viscosity increases on cooling, partly because of the cold agglutination, partly because some casein diffuses out of the casein micelles.
2. *Chemical changes* include uptake of $O_2$ and $N_2$ and loss of $CO_2$. The oxygen may cause oxidation of some lipids and other substances, notably vitamin C. Also, light may induce reactions. Salt composition and pH vary with temperature.
3. *Biochemical changes* are largely enzymatic. Milk contains numerous enzymes. The best-known effects are lipolysis (particularly if the fat globules have been damaged), proteolysis, and hydrolysis of phosphoric acid esters.
4. *Microbial changes* are often the most conspicuous. The main one is souring with all of its consequences, but numerous other changes take place, particularly at higher temperatures (up to 37°C). At room temperature, drastic changes mostly occur within a a day. At 5°C, it may take several days to observe significant effects.

## 1.5. PROCESSING

Processing, of course, is intended to change milk, and it may be argued that processed products should not be called milk. Still, it is common practice to do so. In this book, we will often discuss milk that has been treated in some way. Therefore, we will mention briefly the more common processing treatments.

Heating is nearly always applied, and it induces numerous chemical and other changes, the extent of which depends on temperature and duration of heating. Low pasteurization (e.g., 15 s at 74°C) is a fairly mild treatment that kills most microorganisms and inactivates some enzymes but does not cause many other changes. High pasteurization (e.g., 15 s at 90°C, but varying widely) is more intense; all vegetative microorganisms are killed, most enzymes are inactivated, part of the whey proteins become insoluble, and –SH groups become exposed. Sterilization (e.g., 20 min at 118°C) is meant to kill all microorganisms, including spores; all enzymes are inactivated; numerous chemical changes, such as browning reactions, occur; and formic acid is formed. UHT (ultra-high temperature) heating (e.g., at 145°C for a few seconds) is meant to sterilize milk while minimizing chemical changes; even some enzymes are not inactivated fully.

Separation, usually by means of a flowthrough centrifuge called a cream separator, yields skim milk and cream. The skim milk has a very low fat content, 0.05–0.08%. Milk skimmed after gravity creaming has a much higher fat content. Unless stated otherwise, the term *skim milk* will refer to centrifugically separated milk. By mixing skim milk and cream, milk may be standardized to a desired fat content.

Homogenization of milk leads to a considerable reduction in fat globule size. Such milk creams very slowly but is also altered in other respects. All sterilized milks or, more generally, all long-life liquid milk products, are homogenized in practice.

Evaporation removes water, producing a more concentrated milk. Many properties are altered; for instance, the pH decreases.

Membrane processes may be applied to remove water; this is called reverse osmosis. Ultrafiltration separates milk into a concentrate and a permeate that is rather similar to milk serum. Electrodialysis removes some inorganic salts.

Fermentation or culturing of milk, usually by lactic acid bacteria, causes considerable alteration. Part of the lactose is converted to lactic acid, causing a decrease in pH to such an extent that the casein becomes insoluble. This makes the milk more viscous. The bacteria also produce other metabolites, the kinds and concentrations depending on bacterial species.

## *Suggested Literature*

Several elementary textbooks on dairy chemistry have been published, and texts on food chemistry or dairy technology often include some material on subjects treated in this book. The most complete handbook is

B. H. Webb, A. H. Johnson, and J. A. Alford, eds., *Fundamentals of Dairy Chemistry*, 2nd ed. (Westport: AVI, 1974).

A wide range of subjects is covered by

C. Alais, *Science du Lait, Principes des Techniques Laitières*, 3rd ed. (Paris: SEP Editions, 1974).

A Spanish edition of this book also exists.

# 2

# Biosynthesis and Secretion of Milk

This chapter summarizes the basic features of the processes involved in milk secretion. The purpose is to provide a biological background for considering the peculiarities and variations of milk composition and structure. In no sense is it an exhaustive treatment of the intricacies of biosynthesis of milk constituents or of the physiological processes involved in secretion.

## 2.1. THE MAMMARY GLAND

Milk is a unique product secreted by the mammary gland under complex hormonal control. Milk is synthesized in distinctive secretory cells, which comprise the secretory mammary epithelium. As a result of sequential changes in the hormonal environment, these cells proliferate and develop during pregnancy to be brought into full synthetic activity near the time of parturition. Various hormones, including estrogen, progesterone, and growth hormone, are involved in the structural development of the mammary gland. Insulin promotes cell division of the mammary epithelium, corticosteroids are involved with the development of the cellular organelles for the synthesis of milk constituents, and prolactin is essential for the initiation and maintenance of lactation. Each secretory cell secretes complete milk. Many of the constituents are synthesized in these cells, and others are transported through them.

Figure 2.1 diagrams the essential and distinctive structural features of mammary secretory cells. These cells are arranged around alveoli, as shown in Figure 2.2. In general, precursors enter the secretory cells from the blood circulation on the basal side, and milk is secreted from the apical side into

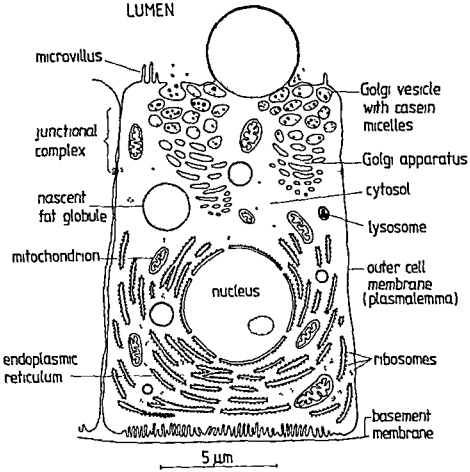



**Figure 2.1.** Diagrammatic representation of mammary secretory cell

the lumen of the alveolus. Myoepithelial cells surround the alveolus. The hormone oxytocin stimulates contraction of these cells, thus squeezing milk out of the alveoli into the ducts. The alveoli are interconnected by an intricate network of ducts that lead to the milk cistern. (See Figure 2.3.) In the bovine gland, at least, a considerable portion of the milk secreted between milkings accumulates in the cistern.

The biosynthetic and physiological processes involved in milk secretion have been elucidated by studies of differences in concentrations of certain constituents between arterial blood supplying the mammary gland and ve-

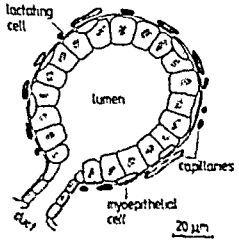



*Figure 2.2. Arrangement of secretory cells around alveolar lumen*

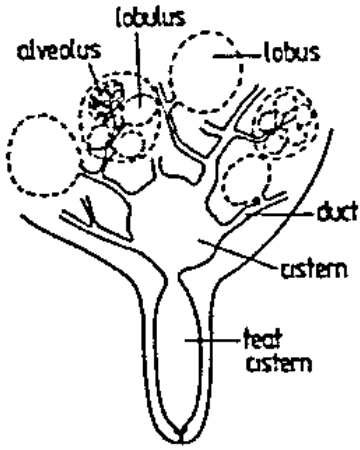



**Figure 2.3.** Gross structure of mammary gland.

nous blood leaving it, by electron microscopy of mammary tissue at various stages, and by enzyme assays and radioactive tracer studies of biosynthetic processes in intact animals, cultures of mammary cells, and cell fractions.

The biosynthetic processes occur at various sites in the cell. Intermediate compounds required for synthesis of protein, lactose, and fat are produced in cytosol and mitochondria; protein and lipid are synthesized in the endoplasmic reticulum; lactose synthesis, posttranslational modification of proteins, and assembly of casein micelles occur in the Golgi apparatus.

The principal function of the mitochondria is the transfer of energy from oxidizable substrates to ATP, which supplies the energy essential for synthesis processes. Citrate is synthesized in the mitochondria and can diffuse through their membranes into the cytosol. The mitochondria also supply carbon for the synthesis of nonessential amino acids.

## 2.2. PRECURSORS OF MILK CONSTITUENTS

Most of the constituents of milk are synthesized in the mammary gland, although some are transferred from blood. (See Table 2.1.) Lactose and the principal milk proteins are organ-specific; they are not synthesized in any tissue other than the mammary gland. The proteins are also species-specific, although they exhibit well-defined taxonomic homology. Milk fat is both species-and organ-specific, particularly regarding its fatty acid composition.

Cattle subsist on diets largely of plant origin. The original precursors of most of the constituents of milk are the cellulose, starch, protein, fat, minerals, and vitamins of the plant materials consumed. Cattle require dietary sources of Ca, Mg, Na, K, P, Cl, Co, Cu, Mo, Fe, Mn, Zn, I, F, Se, S, and vitamins A, D, and E. If the contents of these materials are not sufficient in the plant diet fed to the cattle, the deficiencies must be supplied by additives. Water also is essential for milk production. Dairy cows require 3–4 kg water per kilogram of milk produced.

Major sites of modification of the constituents of the ingested feed are the rumen, the small intestine, and the liver. Rumen microflora hydrolyze

**Table 2.1. Relation between Some Milk Constituents and Their Blood Precursors**

| Milk Constituents | | Precursors in Blood Plasma | |
|---|---|---|---|
| Name | Content g kg $^{-1}$ | Name | Content g kg$^{-1}$ |
| Water | 860 | Water | 910 |
| Lactose | 46 | Glucose | 0 5 |
| Protein | | | |
| casein | 26 | Amino acids | 0 4 |
| $\beta$-lactoglobulin | 3 2 | | |
| $\alpha$ lactalbumin | 1 2 | | |
| lactoferrin | 0 1 | | |
| blood serum albumin | 0 4 | Blood serum albumin | 32 |
| immunoglobulins | 0 7 | Mostly the same | 15 |
| Enzymes | traces | Amino acids, vitamins (in part) | — |
| Fat | | Acetate | 0 1 |
| triglycerides | 38 | $\beta$-hydroxybutyrate | 0 06 |
| phospholipids | 0 3 | Some lipids | 2 |
| Citrate | 1 6 | Glucose | 0 5 |
| Minerals | | | |
| Ca | 1 3 | Ca | 0 1 |
| P | 0 9 | P | 0 1 |
| Na | 0 4 | Na | 3 4 |
| K | 1 5 | K | 0 3 |
| Cl | 1 1 | Cl | 3 5 |

ingested polysaccharides, proteins, and lipids Their fermentative processes produce large amounts of acetate, propionate, and $\beta$-hydroxybutyrate, which are absorbed from the rumen into the bloodstream, and $CO_2$ and $CH_4$, which are vented on eructation These microorganisms saturate (hydrogenate) unsaturated fatty acids from the food fat They synthesize amino acids, including the dietary essential ones, proteins, considerable quantities of B vitamins, and vitamin K The microorganisms themselves are digested in the intestine, and their components are utilized by the animal Dairy cows have been maintained and have produced normal milk on a diet of cellulose, urea as a source of nitrogen, minerals, and vitamins A, D, and E

Proteins are hydrolyzed enzymatically in the small intestine, and the resulting amino acids are absorbed into the blood Fats, likewise, are hydrolyzed by lipases in the small intestine, the resulting fatty acids are reesterified in the intestinal mucosa and transferred to blood via the lymph, largely in

the form of chylomicrons and lipoproteins. In ruminants, little glucose is absorbed from the intestine. Rather, glucose is produced from propionate and amino acids by gluconeogenesis in the liver.

Glucose is the sole precursor of lactose and citrate, and it is the source of part of the glycerol moiety of lipids. The remainder of the glycerol of milk lipids comes from triglycerides supplied to the cell. Acetate, β-hydroxybutyrate, and triglyceride are the sources of fatty acid residues in milk lipids. The dietary essential and some of the nonessential amino acid residues of milk proteins come from amino acids circulating in the blood. The mammary cells have some capacity to synthesize and interconvert certain nonessential amino acids. The ATP required as a source of energy for synthetic processes in the cell is produced by oxidation of glucose and acetate through the usual citric acid cycle and respiratory chain of reactions. Reducing power in the form of NADPH, required for synthesis of fatty acids, is produced from glucose by the pentose–phosphate pathway and by the oxidation of isocitrate, formed in the citric acid cycle, to α-ketoglutarate. Figure 2.4 shows the balance between uptake of precursors and output of milk constituents. This figure presents data from goats, but similar relations no doubt hold for other ruminants, including cows.

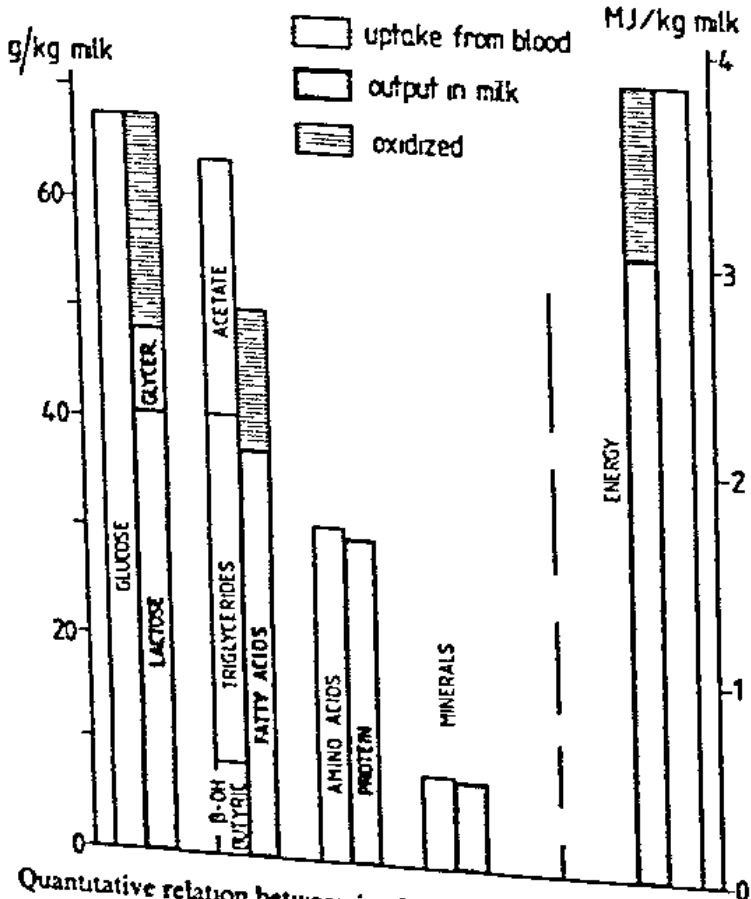



**Figure 2.4.** Quantitative relation between intake of precursors from blood and output of milk constituents. After J. L. Linzell, *Proc. Nutr. Soc.* 27 (1967):44.

Approximately 500 volumes of blood are required to furnish the precursors of one volume of milk. The mammary gland can secrete about 2 g of milk per gram of tissue per day. A gram of tissue contains about $2 \times 10^8$ cells, and thus the output of milk is on the order of $10^{-8}$ g per cell per day.

The energy content of milk is approximately 2.7 $kJ \cdot g^{-1}$, and the daily energy output is about 5.4 $kJ \cdot g^{-1}$ of tissue. Gross energetic efficiency (energy of milk/digestible energy of feed) is about 35% in dairy cows, although some high producers attain levels as high as 48%. Dairy cows require metabolizable energy for maintenance in proportion to the 0.75 power of their body weight. The daily requirement is about 500 $kJ \cdot kg^{-0.75}$. For milk production, they need 1.5 kJ metabolizable energy per kilojoule of milk energy secreted. Thus, a 500-kg animal producing 25 kg of milk daily would require 50 MJ for maintenance and 100 MJ for milk.

## 2.3. BIOSYNTHETIC PROCESSES

### 2.3.1. Lipids

The fatty acids of milk fat originate from three sources:

1. Preformed fatty acids from food fat transferred to the mammary gland via the blood and the lymph in the form of triglycerides and free fatty acids. For the most part, these are acids of 16 or more carbons.
2. Acids synthesized by the gland from acetate and $\beta$-hydroxybutyrate produced by rumen bacteria. Acids produced in this way have short and medium chains ($C_4$–$C_{14}$ and part of the $C_{16}$). Acetate contributes increments to all of the $C_4$–$C_{14}$ acids and $\beta$-hydroxybutyrate is used primarily for the initial four-carbon "primer" unit of most fatty acids synthesized. Mammary synthesis of fatty acids follows the pathway, common in other tissues, via malonyl coenzyme A and successive addition of two-carbon units. The NADPH required for reduction at each step is supplied partly from the degradation of glucose in the pentose–phosphate cycle and partly from oxidation of isocitrate, from the citric acid cycle, to $\alpha$-ketoglutarate. The chain-length distribution of the fatty acids produced differs among species. Evidently, it is controlled by the specificity of the enzymes that remove the fatty acids from the synthetase complex.
3. Acids synthesized from glucose through glycolysis, the citric acid cycle, and cleavage of citrate to form acetyl coenzyme A and oxaloacetate. This is not a significant source of milk fatty acid in ruminants.

Low-roughage diets diminish acetate and increase propionate production in the rumen. Milk produced by cows on low-roughage diets may have only

half the fat content of that from cows on high-roughage diets, and the proportion of short- and medium-chain saturated acids, which are synthesized from acetate, is greatly diminished. This phenomenon is referred to as the "low-fat syndrome."

Rumen microorganisms saturate (hydrogenate) the fatty acids ingested in food to a considerable extent. Thus, circulating lipids and depot fats are highly saturated. If fat encapsulated in formaldehyde-treated casein is fed to cows, it is protected from alteration in the rumen and is released in the abomasum. Feeding a highly unsaturated oil, such as safflower oil, in this way results in a great increase in the $C_{18:2}$ content of the milk fat.

The mammary cells of ruminants have powerful desaturase activity that converts $C_{18:0}$ to $C_{18:1}$ acid. (It also desaturates $C_{16:0}$ to $C_{16:1}$ to some extent.) The desaturase is inhibited by certain cyclopropene acids found in the seed oil of some plants such as *Stercula foetida*. Feeding oil of this kind, encapsulated to protect it from rumen saturases, increases the saturated acid content of the milk fat.

The principal pathway of biosynthesis of triglycerides involves activation of fatty acids by formation of esters with coenzyme A (CoA), reaction of two molecules of such esters with *sn*-glycerol-3-phosphate ($\alpha$-glycerol phosphate) to form phosphatidic acid, cleavage of phosphate by phosphohydrolase to form a 1,2-diacyl *sn*-glycerol (1,2-diglyceride), and, finally, further acylation by another molecule of acyl CoA to form a triglyceride.

In ruminant milk fat, the short-chain ($C_4$ and $C_6$) acyl groups are located almost exclusively in the 3-position of the glycerol moiety. This suggests that the short-chain acids arising from de novo synthesis in the mammary cells are esterified with a 1,2-diglyceride in the last step of formation of milk triglycerides. The abundant palmitate ($C_{16:0}$) group is esterified preferentially in the 2-position of high molecular weight triglycerides and in the terminal (positions 1 and 3) of low molecular weight triglycerides.

The phospholipids phosphatidyl choline and phosphatidyl ethanolamine are formed by reaction of phosphatidic acid with cytidine diphosphate choline or cytidine diphosphate ethanolamine, respectively. Phosphatidyl inositol and phosphatidyl serine, on the other hand, are formed from cytidine diphosphate diglycerides reacting with free inositol or free serine.

### 2.3.2. Proteins

Figure 2.4 indicates that slightly more total amino acids are taken up by the mammary gland than are secreted as milk protein. However, such near equivalence does not hold for each of the individual acids. Some of the essential acids (e.g., methionine, phenylalanine) are indeed taken up in amounts just sufficient to account for their secretion in milk protein. Some others are taken up in excess, and still others in less than the amounts transmitted to milk protein. The mammary cells balance the amino acids absorbed with those secreted by interconversion of one to another and by synthesis from citric acid cycle and glycolytic-pathway intermediates.

All available evidence indicates that the distinctive proteins of bovine milk—caseins, $\alpha$-lactalbumin, and $\beta$-lactoglobulin—are synthesized on ribosomes in the rough endoplasmic reticulum by mechanisms that have been well established for protein synthesis in general. The code determining the sequence of amino acids in proteins is located in genes (DNA molecules) in the nuclei of the cells. The gene serves as a template for synthesis in processes called "transcription" of several types of RNA molecules: messenger (mRNA), transfer (tRNA), and ribosomal (rRNA). Each of these plays a specific role in directing and controlling the synthesis of proteins of determinate sequence. Individual tRNAs are specific for each of the 20 amino acids that can be incorporated into proteins. They covalently bind amino acids in the cell as amino acyl derivatives and convey them to the ribosomal site of synthesis. Ribosomes consist of particles of about 55% rRNA and 45% protein. Each tRNA contains a specific nucleotide triplet or anticodon, which is recognized by and binds to a complementary codon triplet in the mRNA associated with ribosomes. This process brings the amino acid residues attached to tRNA close enough to each other so that peptide bonds can be formed by an enzyme-catalyzed reaction. This process, called "translation," proceeds in a sequential fashion, one amino acid residue after another being attached to the growing polypeptide. Certain codons signal initiation and termination of polypeptide chains.

Some mRNAs specific for some of the milk proteins have been isolated from mammary tissue of a few species and have been shown to be capable of directing synthesis of those proteins in cell-free systems derived from other organisms. Genetically controlled variants occur in several of the bovine milk protein families. These have one or more differences in amino acid sequence as a result of mutations that have occurred in the genes during the history of the species.

Some reactions that may collectively be called "processing" or "post-translational modification" occur in proteins after synthesis. Milk proteins, like other exported secretory proteins, are synthesized with a leader peptide that is later cleaved off by specific proteolysis. This phenomenon has been well demonstrated in the case of $\alpha$-lactalbumin. The leader peptide is concerned somehow with the process of transfer of the protein in and from the rough endoplasmic reticulum to the Golgi apparatus.

Another posttranslational modification is phosphorylation of certain serine and threonine residues in the casein. This event is catalyzed by protein kinases operating in the Golgi apparatus. Phosphate is transferred from ATP to the protein forming a $-CH_2-O-PO_3H$ group. The particular seryl or threonyl groups phosphorylated are determined by the neighboring sequence of residues. The phosphoryl kinase appears to recognize the sequence Ser/Thr-X-Glu/SerP, either Glu or SerP providing an anionic recognition site.

Glycosylation of proteins, catalyzed by glycosyl transferases, also occurs in the Golgi. An N-acetylglucosamine is first attached through its C-1 to either the amide nitrogen of an asparaginyl residue or the hydroxyl oxygen of a seryl or threonyl residue. Additional carbohydrate units (galactosyl, N-

acetylneuraminyl) are then added by transfer from nucleotide sugars to form oligosaccharide units attached to the protein. The κ-casein in bovine milk is glycosylated to varying extents; a small fraction of the α-lactalbumin and a rare variant of the β-lactoglobulin also are glycosylated.

Still another posttranslational modification is formation of disulfide linkages by interaction of two cysteinyl residues either within or between polypeptide chains. The site where the process occurs in the cell is not known. Furthermore, it is not known whether the oxidative process of forming the –S–S–links is catalyzed enzymatically. In cases where two or more intramolecular –S–S–linkages are possible, they are formed between specific cysteinyl residues rather than at random. Evidently, the sequence directs the folding of the polypeptide in order to bring particular thiol groups into juxtaposition where they can react to form –S–S–linkages. α-lactalbumin and β-lactoglobulin are milk proteins with prominent and distinctive disulfide linkages that are important determinants of their structures and properties.

Genetic control of the biosynthesis of milk proteins is attested to by the fact that the total protein content of milk and the proportions of individual proteins vary somewhat within and among breeds of cows. (See Chapter 9.) The protein content increases (but the proportions of protein components remain constant) when diets high in concentrates are fed; these increase the ratio of propionate to acetate produced in the rumen and diminish the production of milk fat. Feeding protein in excess of requirements does not alter the protein content or proportions of components, but it does increase the nonprotein nitrogen content of the milk.

### 2.3.3. Lactose

The biosynthesis of lactose involves coupling a galactose unit to glucose by a β-1,4-glycosidic linkage. The reactions involved are as follows:

$$\text{UTP} + \text{Glc-1-P} \xrightarrow{(\text{EC2 7 7 9})} \text{UDP-Glc} + \text{P-P} \qquad (2.1)$$

$$\text{UDP-Glc} \xrightleftharpoons{(\text{EC5 1 3 2})} \text{UDP-Gal} \qquad (2.2)$$

$$\text{UDP-Gal} + \text{Glc} \xrightarrow{(\text{EC2 4 1 22})} \text{lactose} + \text{UDP} \qquad (2.3)$$

UTP = uridine triphosphate and the nucleotide sugars are uridine derivatives
P-P = pyrophosphate
Glc = glucose
Gal = galactose
EC2.7.7.9 = uridine diphosphate glucose phosphorylase
EC5.1.3.2 = uridine diphosphate glucose-4-epimerase
EC2.4.1.22 = lactose synthase

The basic unit of lactose synthase is a galactosyltransferase not unique to the mammary gland; in other cells, tissues, and organisms, such enzymes

catalyze transfer of galactosyl groups from UDP-Gal to N-acetylglucosamine residues in oligosaccharides attached to glycoproteins. A distinctive feature of mammary cells is synthesis of a unique protein, α-lactalbumin, which so modifies galactosyl transferase that it couples galactosyl groups to free glucose thus forming lactose. This synthesis occurs in the membranes of the Golgi vesicles. The rate of synthesis of lactose at the initiation of lactation (lactogenesis) is certainly controlled by the rate at which α-lactalbumin becomes available, but the rate-limiting reaction(s) and reactant(s) during the further course of lactation have not been identified.

Lactose, being nondiffusible from the Golgi vesicles, draws water into them osmotically and, thus, lactose synthesis largely regulates the volume of milk secreted. A small amount of galactosyl transferase and 1.0–1.2 g of α-lactalbumin per liter are secreted into the milk. The α-lactalbumin contributes significantly to the nutritive value of the milk proteins because of its high tryptophan and cystine contents.

### 2.3.4. Citrate

Citrate is important in milk chemistry because it forms a stable soluble ion with Ca, thus lowering $Ca^{2+}$ activity, and because it is the precursor of aroma compounds produced by certain bacteria.

Citrate is produced in the mitochondria of the cell in the well-known citric-acid cycle of carbohydrate metabolism. It diffuses to the cytosol. Mammary cells of some species (e.g., mice and rats) have in the cytosol a citrate-splitting enzyme, ATP-citrate lyase, which catalyses the reaction of citrate with coenzyme A to form oxaloacetate and acetyl CoA. The latter then can be used for synthesis of fatty acids. Ruminants and some other species, having very low ATP-citrate lyase activity in the mammary cells, cannot utilize glucose for synthesis of fatty acids and, thus, citrate accumulates in the milk.

The citrate content of cow's milk declines somewhat during the course of the lactation period; it is slightly depressed when high-concentrate diets are fed, leading to the low-fat syndrome.

## 2.4. TRANSPORT PROCESSES

In normal healthy mammary tissue, tight junctions between the secretory epithelial cells prevent direct passage of materials between them. Thus, substances transferred from blood to milk must pass through the cells themselves. Furthermore, precursors gain entrance through the basal membrane, and synthesized products leave through the apical membrane. Both active (movement against a concentration gradient) and passive (movement down a concentration gradient) transport are involved.

Mastitic milk and milk from late lactation have higher than normal concentration of such blood components as serum albumin, certain lipoproteins,

plasmin, leukocytes, $Na^+$, and $Cl^-$. The normally tight junctions are evidently somewhat "leaky" in these conditions, allowing a paracellular transfer of blood constituents into the milk.

An important feature of the function of mammary cells is the formation of Golgi vesicles within which lactose is synthesized, proteins accumulate and are "processed," and casein micelles are formed. (See Figure 2.1.) These vesicles migrate to the apical membrane, fuse with it, and then open to the alveolar lumen to discharge their contents (exocytosis).

Fat droplets migrate through the cell separately from the Golgi vesicles, although some of the latter may become attached to the droplets. The forces responsible for migration of Golgi vesicles and fat globules have not been well defined, but probably the network of microtubules within the cell is involved in the process.

### 2.4.1. Ions and Small Molecules

The molar ratio K/Na is 0.05 in blood and 2.20 in milk. Such a change in concentration suggests that a Na–K "pump" operates in the basal but not in the apical membrane of the cell. Such pumps involve a $Na^+K^+$-ATPase in the membrane. This acts on internal Mg ATP and is stimulated by $Na^+$ from the inside and by $K^+$ from the outside. Three $Na^+$ ions leave and two $K^+$ ions enter the cell for every molecule of ATP hydrolyzed to ADP and phosphate. An ATPase of this kind has been found in mammary cell preparations. The chloride concentration of milk is ~ 30 m*M*; that of blood is ~ 100 m*M*. The apical membrane of the cell is permeable to $Cl^-$, but the concentration of chloride is greater in the cell than in milk. The mechanism by which this imbalance is achieved is not known with certainty, although both active transport (pump) and osmotic waterflow have been suggested. Calcium probably enters the cell by passive diffusion, but its accumulation into the Golgi vesicles for the formation of casein micelles likely involves a Ca pump (i.e., a $Ca^{2+}$-stimulated ATPase). Little is known of the mechanism by which phosphate crosses the basal membrane or enters the Golgi vesicles. Likewise, the route by which citrate is secreted is not known. It is synthesized in the mitochondria and is transported from there into the cytosol, but whether, to what extent, and how it enters Golgi vesicles is not known.

### 2.4.2. Proteins

The proteins synthesized in the mammary cells—caseins, $\beta$-lactoglobulin, and $\alpha$-lactalbumin—are transported to the apical membrane via Golgi vesicles and discharged into the lumen by exocytosis.

The major immunoglobulins found in the milks of all species are IgG, IgA, and IgM. The principal kind found in bovine colostrum and milk is IgG1, but smaller amounts of IgG2 are also present. They are synthesized

elsewhere in the body and transported via the blood and the mammary gland. IgA and IgM, present in bovine colostrum and found in much greater amounts in the colostrum and milks of other species, are produced by plasma cells (specifically activated lymphocytes) located in the mammary gland adjacent to the secretory epithelium. The routes by which these immunoglobulin molecules enter milk are not yet understood completely. Current evidence favors a transport route through the secretory cells that is highly specific for each class of immunoglobulin.

Little attention has been paid to the mode of entrance of serum albumin into milk. It has been found in milks of all species examined for it; bovine milk contains 0.2–0.4 g·liter$^{-1}$. It seems unlikely that it enters milk by "leakage" between secretory cells, except in cases of severe mastitis when its concentration in milk becomes much greater.

### 2.4.3. Fat Globules

Triglycerides are formed within the rough endoplasmic reticulum. The size distribution of the fat globules in milk indicates that two populations are present. (See Section 14.1.) One consists of numerous small globules below ~ 1 $\mu$m in diameter and accounts for only 2% of the fat. The second, accounting for most of the fat, has a range of globular diameter of about 1–10 $\mu$m. These populations may well have different synthetic histories. Globules of the second population obviously grow as they progress through the cell, and this growth probably arises from continuing synthesis of triglycerides at their surfaces. The globules migrate to the apical membrane of the cell, probably acquiring a surface layer of phospholipid on the way. Upon contact with the membrane, they are enveloped, the membrane is pinched off at the base, and the globule thus formed is released into the lumen. The cell membrane consists principally of glycoproteins and polar lipids; it contains numerous enzymes.

Some Golgi vesicles may become attached to the globules and their membranes discharged along with the fat globules. Also, a tiny amount of cytoplasm (comprising about 1 ppm of the milk) may be released into the lumen when the fat globule leaves the cell. The globules may remain in contact with the cell membrane for a fairly long period as compared to their stay in the cytosol. The original membrane of the milk fat globule, therefore, is essentially the apical plasma membrane of the cell disposed in the same orientation (same side out) that it had in the cell. It undergoes modification later. The plasma membrane lost from the cell by enveloping the fat globules is replaced by membranes of Golgi vesicles that have discharged their contents. Thus, a flow of membrane seems to occur from endoplasmic reticulum to Golgi vesicles to apical plasma membrane. One can calculate readily, however, that only about 1% of the membrane of the Golgi vesicles would be needed to cover the fat globules.

## 2.5. CELLS AND CELL FRAGMENTS IN MILK

Bovine milk may contain $5 \cdot 10^4$–$5 \cdot 10^5$ cells per milliliter. Although they were long considered to be mostly secretory epithelial cells, recent electron microscopic investigations show them to be mostly leukocytes, predominantly macrophages in normal milk, and polymorphonuclear leukocytes in colostrum. These cells engulf some fat globules and casein micelles in milk. Lymphocytes are also present, but no secretory epithelial cells and only a few ductal epithelial cells. Leukocytes contain numerous enzymes; catalase is especially prominent. They are also the main source of nucleic acids in milk. The population of leukocytes is much enhanced in milk from cows with mastitis. The macrophages in normal milk probably originate from the monocyte population of the alveolar and ductal epithelium, but their route into the milk has not been established. Evidently, they function to protect the maternal udder and the gut of the suckling from invading bacteria. Human and rat milk carry considerable populations of lymphocytes, and there is evidence that these can establish themselves as functioning producers of immunoglobulins in the sucklings consuming them, but this phenomenon has not been demonstrated for ruminants.

A number of kinds of membrane fragments have been detected in ruminant skim milk. These include secretory vesicles and endoplasmic reticulum, vesicles derived from cell membrane, fractions of fat globule membrane, and possibly microvilli and membrane-enclosed viruses. Such fragments can be isolated as "cytoplasmic droplets" or "milk microsomes" by appropriate centrifugal techniques. They carry various enzyme activities and have the capacity to oxidize acetate and glucose to $CO_2$, to convert acetate to fatty acids, to desaturate saturated fatty acids, to convert glucose to glycerol, and to synthesize triglycerides and phospholipids.

## 2.6. LIMITATIONS ON MILK COMPOSITION

The composition is limited by certain physical properties. One such limitation is the fact that milk is isoosmotic with blood. In both fluids, the sum of concentrations of solutes is about 0.3 $mol \cdot kg^{-1}$ of water. The principal contributors to the osmotic pressure of blood are Na and Cl ions, but in milk lactose, K, Na, and Cl all contribute. (See Table 4.4.) As mentioned earlier, the biosynthesis of lactose is the principal regulator of the water content of milk. Variations in the lactose content of cow's milk are small, but those that do occur are correlated directly with $K^+$ and inversely with $Na^+$ and $Cl^-$; the total osmolality is maintained within narrow limits.

A second limitation on milk composition is the solubility of calcium phosphate and calcium citrate. Bovine milk, saturated with both, contains in solution about 10 mmol each of Ca, inorganic phosphate, and citrate per

liter Additional calcium and phosphate, of great nutritive advantage to the nursling, are carried out in the form of a colloidal complex with casein

A third physical limitation on milk composition is that the final melting point of the milk fat must not be higher than body temperature (37°–40°C) Fats that are liquid at body temperature are digested more readily Furthermore, crystallization of fats of higher melting points might occur in the cells and alveoli, and this might cause, for instance, partial coalescence of fat globules in the udder Ruminants control the melting point of milk fat by synthesizing short fatty acid chains, by desaturating saturated $C_{18:0}$ acids arriving from the blood, and by positioning the acids asymmetrically on the glycerol moiety

## 2.7. CHANGES IN MILK IN DUCTS AND CISTERNS

In mammals like ruminants, which do not nurse their young continuously, the ducts and milk cisterns of the mammary gland have the capacity to hold considerable milk With domestic cattle, the milk drawn at a given milking will have been discharged into the lumens of the alveoli during the preceding 8 to 12-hour period The walls of the ducts are relatively impermeable to the milk constituents, and thus the composition is not altered by selective resorption

Alterations that probably do occur are as follows

1. Reorientation and fosses and gains of materials by the fat globule membrane (See Section 5 3 1 )
2. Proteolytic cleavage of $\beta$ casein by milk plasmin to form $\gamma$-casein and peptide fragments (See Section 6 3 4)
3. Lipolysis of milk fat (See Section 5 5 ) This probably is minor, the free fatty acid content of freshly secreted milk is low and most likely does not originate by lipolysis of triglycerides
4. Biosynthetic processes mediated by "cytoplasmic droplets ' (See Section 2 5 )
5. "Filtering" of fat globules Fat globules are restrained in the ducts of a full udder The fat content of milk increases during milking, and milk with a higher fat content is obtained at a milking following an incomplete one
6. Coalescence of fat globules A few unusually large fat globules occur in milk They may arise by coalescence
7. Contamination with bacteria Milk secreted by healthy udders is sterile but may become contaminated with bacteria as it leaves the udder through the teat canal

## *Suggested Literature*

All aspects of biosynthesis and secretion of milk are covered by

B. L. Larson and V. R Smith, eds., *Lactation: A Comprehensive Treatise*, Vols. 1–4, (New York: Academic Press, 1974–1979).

A brief treatment is given by

T. B. Mepham, "The Secretion of Milk," *Studies in Biology* No 60 (London: Edward Arnold, 1976).

# 3

# Carbohydrates

The most prominent carbohydrate of milks of most species is lactose. It is virtually unique to milk, having been found elsewhere only in fruits of certain members of the *Sapotaceae*. Biosynthesis of lactose by the mammary gland was described in Section 2.3.3.

The concentration of lactose ranges from none in milks of certain seals to about 100 g·liter$^{-1}$ in those of some primates. Bovine milk usually contains more lactose than any other solid constituent, with a rather constant concentration between 45 and 50g·liter$^{-1}$. Other carbohydrates, present in much lower concentrations, are mentioned in Section 3.4.

## 3.1. CHEMICAL PROPERTIES OF LACTOSE

Chemically, lactose is a disaccharide consisting of one residue each of D-glucose and D-galactose joined in a $\beta$-1,4-glycosidic linkage. Both moieties occur predominantly in the pyranose ring form. Thus, the compound is properly called 4-O-$\beta$-D-galactopyranosyl-D-glucopyranose. Its molecular structure is depicted in Figure 3.1.

Chemical reactions of lactose involve the hemiacetal linkage between carbons 1 and 5 of the glucose moiety, the $\beta$-1,4-glycosidic linkage between the sugar rings, the hydroxyl groups, and the –C–C– bonds within the rings. The hemiacetal structure gives rise to an equilibrium between the two anomers, $\alpha$- and $\beta$-, which differ in steric configuration of the –OH and –H around C-1, as depicted in Figure 3.1. These anomers differ in specific optical rotation and in solubility, as discussed in Section 3.2. The dynamic equilibrium between the anomers involves opening and closing the hemiacetal ring, and at any time a small amount of the free aldehyde form is present. This can undergo reactions typical of aldehydes and, because of the equilibrium,

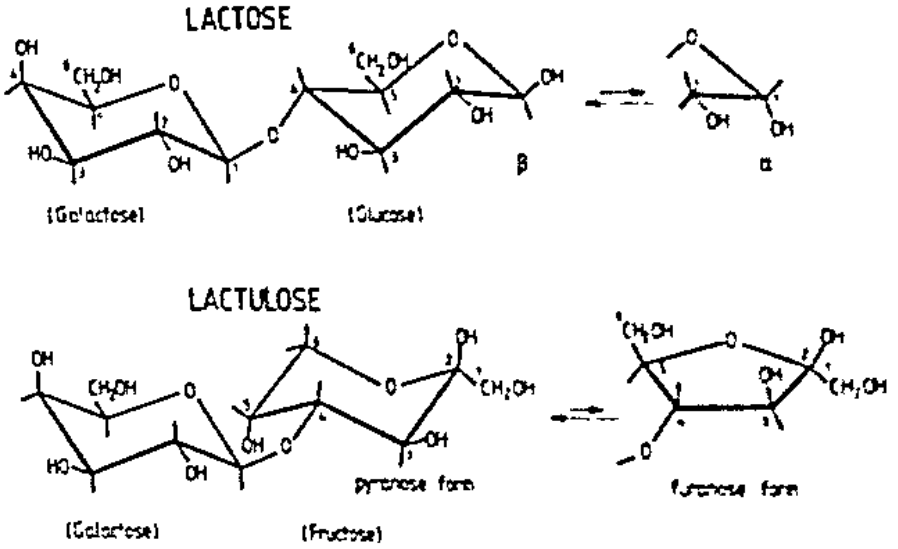



**figure 3.1.** Chemical structure of lactose and lactulose

the entire amount of lactose in a system can react this way. Lactose is thus a reducing disaccharide.

Lactose is not as sweet as such other common sugars as sucrose, fructose, and glucose. It is relatively sweeter at higher concentration. Thus, for example, 1%, 5%, 10%, and 20% sucrose solutions are equivalent in sweetness to 3.5%, 15%, 20%, and 33% lactose solutions. β-lactose is sweeter than α-lactose, but this difference is not important since the small difference between freshly prepared solutions is eliminated quickly by equilibration of the anomers.

Mild oxidation of lactose with such reagents as alkaline copper, iodine, or picrate forms lactobionic acid (4-O-β-D-galactopyranosyl-D-gluconic acid), which has a carboxyl group at C-1 of the glucose unit. Such oxidation reactions often are used to determine reducing sugars quantitatively. (See Section 20.5). Lactose also can be oxidized to lactobionic acid by mild chemical dehydrogenation and by lactose dehydrogenase produced by certain species of bacteria of the genus *Pseudomonas*. Lactobionic acid readily forms 1,4 and 1,5 lactones by esterification of the carboxyl with hydroxyls at C-4 or C-5. Somewhat more vigorous oxidation of lactose with dilute nitric acid ruptures the glycosidic linkage and produces dicarboxylic acid derivatives of the two sugars. The dicarboxyl derivative of galactose, galactaric or mucic acid, was formerly much used as a crystalline derivative for identification of galactose.

Biological oxidation of lactose to $CO_2$ and water can be brought about by mixed cultures of bacteria and protozoa obtained from sewage sludge. Such processes are useful in decomposing lactose-containing wastes from dairy factories.

With gentle reduction, only the free aldehyde of lactose reacts to become an alcohol group, and lactitol (4-O-β-D-galactopyranosyl-D-glucitol) is formed. More drastic reduction splits the glycosidic linkage and various –C–C– bonds and results in the formation of a mixture of alcohols.

### 3.1. Chemical Properties of Lactose

Lactose may be hydrolyzed to D-glucose and D-galactose with mi[illegible] acids, with cation exchange resins in the acid form, and with β-galactosi[illegible] (often called *lactases*). The β-1,4 linkage between the two sugar residues is much more resistant to hydrolysis than is the 1,2 linkage between glucose and fructose in sucrose. Treatments such as an hour at 90°C with 1.5 *M* HCl, or at 150°C with 0.1 *M* HCl, are required to hydrolyze lactose completely.

Sources of β-galactosidase (EC 3.2.1.23) are distributed widely among such diverse organisms as yeasts, bacteria, plants, and mammalian intestinal mucosa. β-galactosidases are highly specific for the galactopyranose structure and the β-1,4 linkage. Some reports have been made of the presence of β-galactosidase in milk, but these have not been verified.

β-galactosidases not only act as hydrolases, but they also can transfer galactose units to other hydroxyl-containing compounds such as glycerol. Acting on lactose, they produce significant quantities of oligosaccharides containing glucose and galactose linked in various ways, some of which involve carbons 3 and 6 of galactose. Preparations of β-galactosidases have been used to lower the lactose content of certain dairy products in order to avoid undesired crystallization. They also have been employed in the manufacture of low-lactose products for people who cannot tolerate lactose because of a lack of sufficient intestinal β-galactosidase. (See Section 19.2).

Upon being heated in solution, lactose, like other sugars, decomposes to yield a mixture of products in proportions depending on concentration of sugar, time, temperature, and pH. Some of the products identified in lactose solutions heated at temperatures of 100°C or above in the absence of amino compounds are 5-hydroxy-2-furfural, furfuryl alcohol, acetol, methylglyoxal, acetaldehyde, formaldehyde, and acetic, formic, lactic, pyruvic, and levulinic acids. These compounds are themselves rather reactive and can yield various polymers, some of which are brown and have distinctive flavors. Aspects of the formation of acid upon heating milk are discussed in Section 10.1.

Heating lactose in the presence of amino compounds induces the very important Maillard reaction that involves formation of a Schiff base between the amino and the aldehyde group of the sugar. The initial reaction product undergoes a whole series of rearrangements and further reactions leading to brown color and off-flavors important in dairy manufacturing processes. The chemistry of these reactions is discussed in Section 10.4, and their relation to flavor of dairy products in Section 18.3.

A compound found in heated milk products is lactulose (4-0-β-D-galactopyranosyl-D-fructose, in Figure 3.1; the fructose moiety occurs predominantly in the pyranose, partly in the furanose form). Concentrations of this compound up to about 1% may occur in commercial evaporated milk (10% of the total lactose). The mechanism by which lactulose is formed from lactose is not known with certainty, but it has been suggested that it could arise by either a Lobry de Bruyn transformation or by rearrangement and splitting of a Schiff base formed between lactose and an amino compound. Trace amounts of epilactose (4-0-β-D-galactopyranosyl-D-mannose) are also

formed on heating milk. Lactulose is somewhat sweeter and considerably more soluble than lactose. It has been alleged to promote the growth of *Bifidobacterium bifidum* and, thus, to be beneficial in the diets of human infants. It has not been accepted or employed widely for this purpose.

## 3.2. PHYSICOCHEMICAL ASPECTS OF LACTOSE

This section deals with the mutarotation, solubility, and crystallization of lactose and the properties of its crystals.

### 3.2.1. Mutarotation

Sugars, having asymmetric carbon atoms, are optically active; their solutions rotate the plane of polarization of a polarized light wave that passes through them either clockwise (dextrorotation, +) or counterclockwise (levorotation, −). (Incidentally, the prefixes D- and L- designate configuration, not direction of rotation.) Each sugar has its characteristic specific rotation, which is the angle, either positive or negative, by which the plane is rotated per unit concentration and per unit path length. Rotation may be observed and measured in a polarimeter, and this furnishes a useful method for determining concentration. Specific rotation depends on temperature and wavelength. Values for some compounds are given in the appendix, Table A.17.

α- and β-lactose differ in specific rotation. A freshly made solution of either will change in rotation with time as equilibration with the other form occurs. This change in rotation and the conversion of one form to the other in solution are called mutarotation. The equilibration involves two reactions:

$$\alpha\text{-lactose} \rightarrow \beta\text{-lactose, reaction constant } K_1$$
$$\beta\text{-lactose} \rightarrow \alpha\text{-lactose, reaction constant } K_2$$

Both are first order reactions. If $a$ and $b$ are the concentrations of α- and β-lactose, respectively, we have at equilibrium (i.e., after infinite time)

$$R = \frac{b_\infty}{a_\infty} = \frac{K_1}{K_2} \tag{3.1}$$

The mutarotation rate can be defined as

$$K = K_1 + K_2 \tag{3.2}$$

This implies that, in the absence of equilibrium, the proportion of the mutarotation that is still to occur is given by $1 - e^{-Kt}$. Examples are given in Figure 3.2. Change of concentration of α-lactose with time is given by

$$\frac{(a - a_\infty)}{(a_0 - a_\infty)} = e^{Kt} \tag{3.3}$$

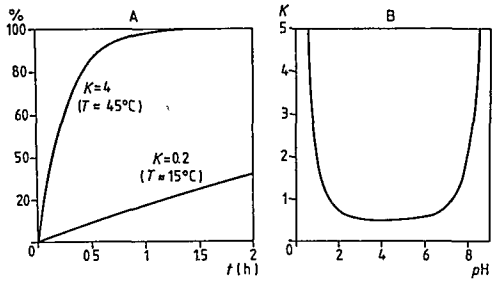



**Figure 3.2.** Mutarotation in lactose solutions. (A) Course of the reaction (% finished) as a function of time $t$; (B) effect of pH on mutarotation rate constant ($K$ in $h^{-1}$). After Troy and Sharp, *J. Dairy Sci.* **13** (1930):140.

where subscripts 0 and $\infty$ refer to time, and similarly for $\beta$-lactose (*b*). If we want to know the amount of, say, $\alpha$ in a lactose mixture, a sample has to be dissolved and then mutarotation causes the $\alpha$ content to change. Equation 3.3 can be used to obtain $a_0$ by extrapolation to zero time; the intercept of a plot of $\ln(a - a_\infty)$ versus $t$ gives $\ln(a_0 - a_\infty)$. See the appendix for a formula directly using optical rotation.

$K_1$ and $K_2$, and thus $K$ and $R$, depend on conditions. Results reported for these parameters in the literature are not fully consistent. The equilibrium ratio in dilute lactose solutions is roughly

$$R = 1.64 - 0.0027\,T \qquad (3.4)$$

where $T$ is in degrees celsius. Thus, a change in temperature causes mutarotation. $R$ decreases with increasing lactose concentration, if the latter is above some 60% by weight. For very high concentrations, as in the amorphous lactose in milk or whey powder, $R \approx 1.25$, independent of temperature, but it may take a long time to attain equilibrium.

At 20°C in pure lactose solutions, $K \approx 10^{-4}\ s^{-1}$, and it increases roughly by a factor of 1.11 per degree Kelvin rise in temperature ($Q_{10} \approx 2.8$ at room temperature; apparent activation energy $\approx$75 kJ·mol$^{-1}$). At room temperature, it takes many hours before equilibrium is reached, at 70°C a few minutes. Several substances affect $K$. Figure 3.2 shows the effect of pH. The salts in milk increase $K$ by a factor of almost 2. Mutarotation rate decreases very much at high sugar concentrations, as, for example, in the high concentration of sucrose in sweetened condensed milk. In skim milk powder containing 2% water, it will take months at 25°C to obtain equilibrium, hours at 50°C, and minutes at 80°C; for a water content of 6%, equilibration takes days at 25°C and about 1 h at 50°C. It should be realized that mutarotation may occur during oven drying of dried milk products or even pure lactose.

### 3.2.2. Solubility

As seen in Figure 3.3, α- and β-lactose differ considerably in solubility and in the temperature dependence of solubility. If α-lactose is put in water, much less can dissolve at the outset than later. This is because of the mutarotation; α-lactose is converted into β-, hence the α-concentration diminishes and more α- can dissolve. At low temperatures, it may thus take a long time before the final solubility is reached; the mutarotation rate is determinant. If β-lactose is put into water, more may dissolve at the outset than later; at temperatures below 65°C, a saturated solution of β-lactose yields on mutarotation more α-lactose than can stay dissolved, and the latter will partly crystallize. The final solubility is identical whether we dissolve α- or β-lactose. Below 93.5°C, it is $R + 1$ times the solubility of α-lactose. Above 93.5°C, β-lactose determines the final solubility and it is $1 + 1/R$ times the solubility of β-lactose. Consequently, if lactose crystallizes from solutions like milk below 93.5°C, α-lactose usually is formed; above 93.5°C, β-lactose usually crystallizes.

Lactose solutions can be supersaturated easily, or, in other words, nucleation does not occur easily. This is indicated roughly in Figure 3.3. At concentrations over 2.1 times the final solubility, spontaneous crystallization occurs, probably because of homogeneous nucleation. (See Section 5.7.2.) At a relative supersaturation below about 1.6, seeding with lactose crystals usually is needed to induce crystallization, unless we wait very long; the solution is thus metastable. In the intermediate range, crystallization depends on several factors, such as composition and time.

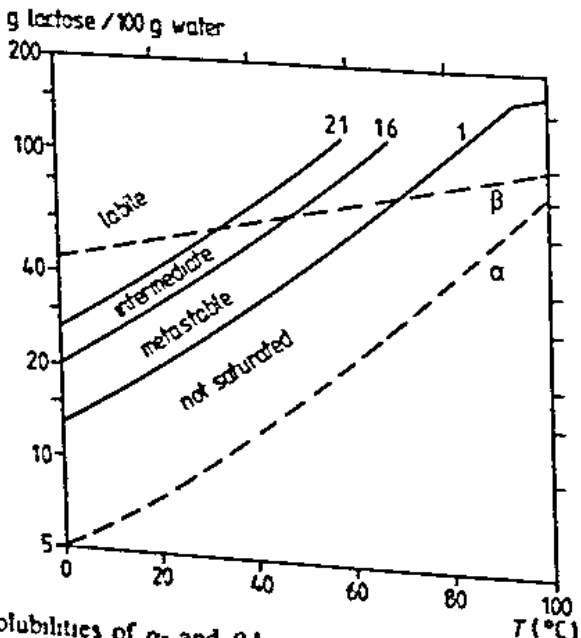



**Figure 3.3.** The solubilities of α- and β-lactose, the final solubility of lactose (line 1), and supersaturation by a factor 1.6 and 2.1, respectively (α-lactose excluding water of crystallization). From various sources.

Crystallization of lactose is of great practical importance, not only as a step in the manufacture of lactose, but also because it may crystallize in some milk products, notably sweetened condensed milk and ice cream. As mentioned above, nucleation is often slow; this implies that few crystals are formed and these become large. If crystals more than 10 $\mu$m in size are present, they can be felt in the mouth, and the product is said to be sandy. We usually have to add numerous tiny seed crystals before crystallization to prevent the development of sandiness. A further complication is the mutarotation. If a lactose solution is brought to crystallize at a low temperature, crystal growth is prolonged because removal of $\alpha$-lactose from the solution is followed by conversion of $\beta$- to $\alpha$-lactose; hence, the solution can stay at a low supersaturation for a long time. Other conditions (crystal size, composition, temperature) being equal, the rate of crystal growth is often about proportional to the square of the absolute supersaturation (concentration minus solubility of $\alpha$-lactose).

### 3.2.3. Crystal Forms

Usually, $\alpha$-lactose crystallizes as a hydrate containing equimolar amounts of lactose and water. The crystals are fairly hard and not hygroscopic. Above 93.5°C, anhydrous $\beta$-lactose crystallizes. $\beta$-lactose dissolves much faster than $\alpha$-lactose hydrate at room temperature, as its solubility is about 10 times higher and the crystals are usually smaller with a larger surface area.

Amorphous lactose is formed when a solution (e.g., milk) is dried rapidly, as in a spray drier, or frozen. It often is called a "glass," but this is not strictly correct. It is a very concentrated solution and it quickly dissolves or, rather, is diluted, on addition of water; but then, $\alpha$-lactose hydrate may start to crystallize. If the water content of the amorphous lactose is low, say 3%, crystallization may be postponed almost indefinitely; nucleation rate is negligible because of the extremely high viscosity of the "solution." The product is, however, very hygroscopic, and when moisture content rises to about 8%, $\alpha$-lactose hydrate starts to crystallize. This is a way to make very small crystals. But when crystallization of lactose caused by moisture uptake occurs in milk or whey powder, the result is caking; powder particles are cemented together by crystalline lactose, forming large and stony lumps.

Some other crystal forms may occur. Figure 3.4 gives a survey, including the transitions. It thus gives the methods for preparing the different forms. In practice, it is almost impossible to obtain pure crystals. For instance, $\alpha$-hydrate usually contains a few percent of $\beta$-lactose, and vice versa.

The different forms mentioned are different crystal polymorphs (i.e., they have different crystal lattices). The properties are thus different. (See also the appendix, Table A.17.) For example, the stable anhydrous $\alpha$ form, also called S lactose, is not hygroscopic but is quite soluble in water, about four times as much as $\alpha$-hydrate. But the latter soon starts to crystallize from a solution of S lactose. The unstable anhydrous $\alpha$ form is very hygroscopic and has the same solubility as $\alpha$-hydrate.

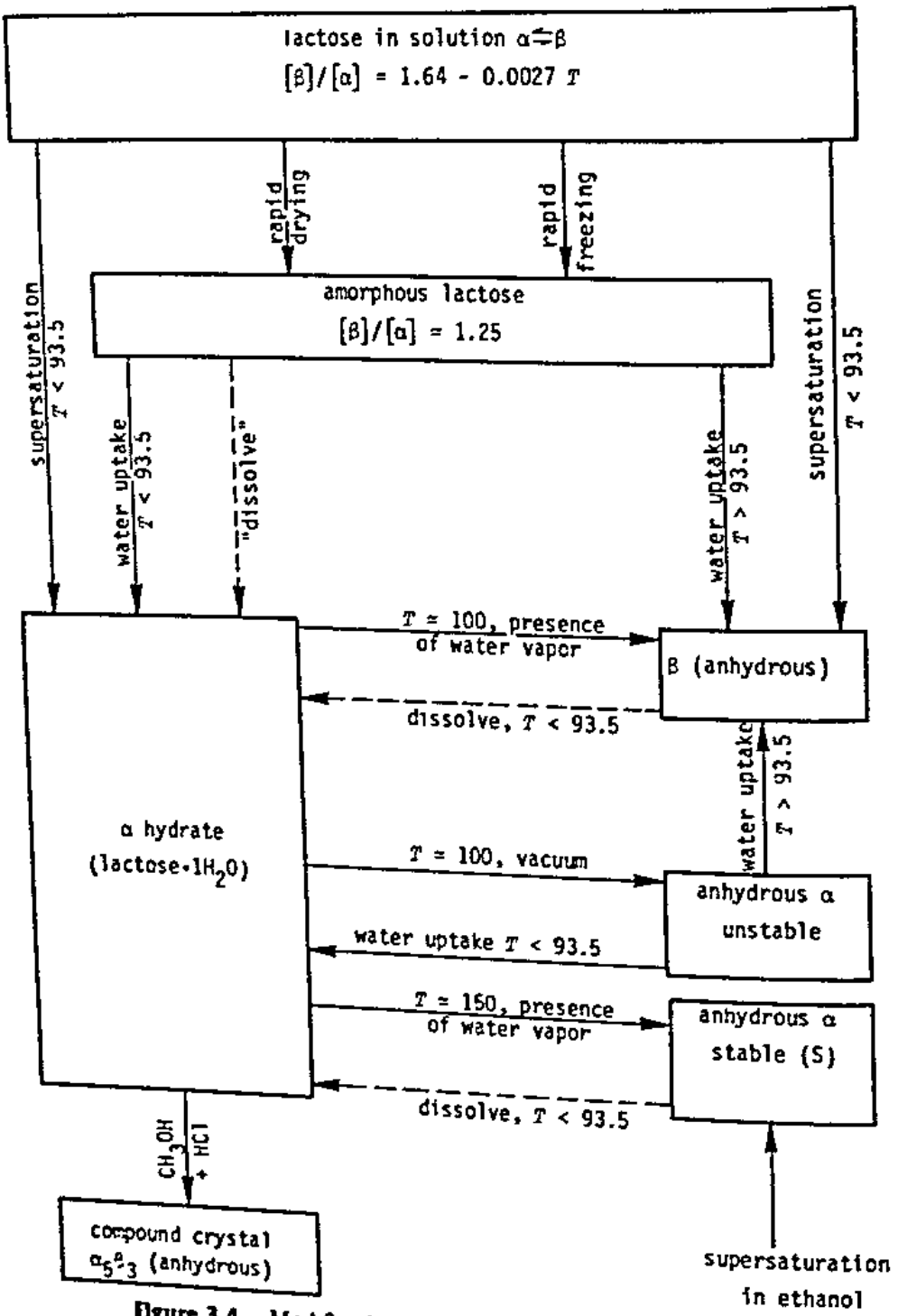



**Figure 3.4.** Modifications of lactose. ($T$ is temperature in °C.)

### 3.2.4. α-lactose Hydrate

α-lactose hydrate is the common form of crystalline lactose. The crystals can have very different shapes, but this is purely a matter of variation in morphology (habit); the crystal lattice is always the same. The commonest shape is the "tomahawk," depicted in Figure 3.5. The figure also gives the Miller indices of the various faces. Several substances inhibit the growth;

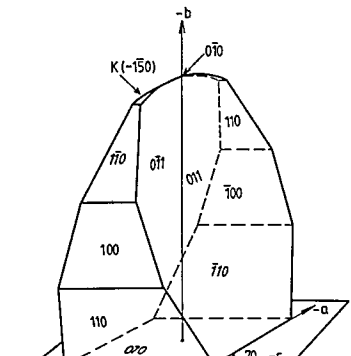



**Figure 3 5.** A common shape of an $\alpha$ lactose hydrate crystal The crystallographic axes and the Miller indices of the faces are given

as their effect differs widely between different faces, the great variety in crystal habit is easily explained (See Table 3 1 ) Some potent inhibitors, such as riboflavin, are present in milk There are many others, besides those mentioned in Table 3 1

Two inhibitors need special consideration $\beta$-lactose has a large inhibitory effect, and under most conditions it is present in a high concentration In special experiments, $\beta$-lactose content can be lowered considerably, and the result on crystal growth is spectacular (See, for instance, Figure 3 6 ) Usually, faces 0$\bar{1}$1 and 0$\bar{1}\bar{1}$ do not grow at all unless supersaturation is extremely high But if only little $\beta$-lactose is present, these faces grow much faster than all other faces, consequently, needle crystals are formed in the direction of the $c$ axis Face 0$\bar{1}$0 never grows in the presence of $\beta$ lactose Consequently, the apex of the crystal as depicted in Figure 3 5 is also the point where the crystal started to grow If supersaturation is high, the slightly curved K faces ~ 1$\bar{5}$0 grow until a 0$\bar{1}$0 face is formed, and growth stops there It appears as if $\beta$-lactose molecules fit well into the 0$\bar{1}$0 and 0$\bar{1}\bar{1}$ faces of $\alpha$ hydrate but then prevent any further uptake of $\alpha$ molecules This, then, largely explains the peculiar shape of the crystal Some other saccharides cause a similar but lesser inhibition

If $\alpha$ lactose hydrate is purified by recrystallization, its rate of crystal growth decreases (See Table 3 1 ) Moreover, the pH of the lactose solution falls It appears that a crystal growth inhibitor is present, one or more lactose monophosphate (4 0 $\beta$-D galactose-phosphate-D-glucose), its concentration in milk is roughly 15 mg liter$^{-1}$ It apparently has a stronger affinity for the $\alpha$ hydrate crystal than $\alpha$ lactose itself But it retards crystal growth consid-

**Table 3.1. Examples of the Rate of Growth of Some Faces (as Indicated by their Miller Indices) of α-Lactose Hydrate Crystals as a Function of Liquid Composition.**

| Supersaturation (%) | Remarks on Composition | Growth ($\mu m \cdot h^{-1}$) of Face | | | | |
|---|---|---|---|---|---|---|
| | | 010 | 110 | 100 | $1\bar{1}0$ | $0\bar{1}1$ |
| 55 | – | 3.8 | 3.3 | 1.3 | 0.3 | 0 |
| 55 | + 10 ppm gelatin | 1.2 | 1.0 | 1.0 | 0.4 | |
| 55 | + 100 ppm riboflavin | 2.7 | 0.0 | 0.0 | 0.0 | 0 |
| 55 | + 10 ppm TMODAC[a] | 0.0 | 0.5 | 0.6 | 0.0 | |
| 120 | – | 43 | 34 | 21 | 12 | 7 |
| 55 | own pH (~ 4) | 3.2 | 2.7 | 1.6 | 0.4 | 0 |
| 55 | pH 7 | 6.6 | 5.0 | 2.7 | 1.2 | |
| 55 | 3 × recrystallized | 0.2 | 0.7 | 1.3 | 0.5 | |
| 55 | Nonionic[b] | 19.1 | 9.1 | 3.1 | 1.2 | |
| 55 | Nonionic + LMP[c] | 0.0 | 0.0 | 0.9 | 0.5 | |

*Source:* After Michaels and Van Kreveld, *Neth Milk Dairy J* 20 (1966):163; and Visser, *Ibid.* 34 (1980):255.

*Note:* Unless stated otherwise, solutions are analytical grade α-hydrate dissolved in water and brought to mutarotation equilibrium.

[a]Trimethyl octadecyl ammonium chloride.

[b]Solution passed through an anion exchanger

[c]Lactose monophosphate.

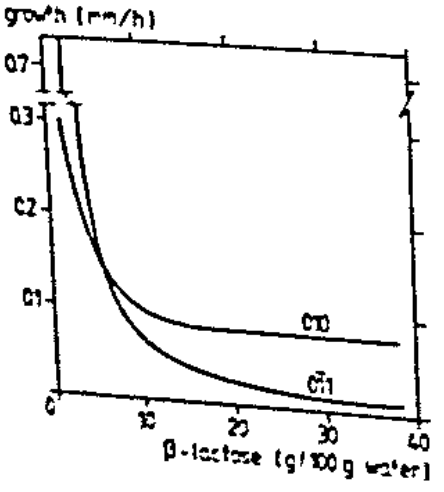



**Figure 3.6.** Effect of the concentration of β-lactose on the growth rate of some faces of an α-lactose hydrate crystal. Supersaturation of α-lactose is 170%. After A. v. Kreveld, *Neth. Milk Dairy J.* 23 (1969):258.

erably, as shown in Table 3.1. It particularly inhibits at low supersaturation. If it is not present, spontaneous (though probably still heterogeneous) nucleation may occur at a much lower supersaturation, say 10% instead of 100%. Several other inhibitors, such as the TMODAC mentioned in Table 3.1, only cause inhibition if the lactose monophosphate is present, not if the latter has been removed by ion exchange.

It is clear now why lactose crystallizes so slowly even if growth is not retarded by slowness of mutarotation. Besides the difficulty of fitting the asymmetric molecules into the crystal lattices, there is considerable competition by β-lactose, and for some faces even strong inhibition. Moreover, some minor components of milk, notably the lactose monophosphate, cause considerable inhibition. Rate of crystal growth is usually not limited by diffusion, except in extremely concentrated solutions like amorphous lactose.

## 3.3. FERMENTATION

Lactose is metabolized by various microorganisms to compounds of lower molecular weight. In the dairy field, the most important fermentations by far are those that produce lactic acid, although propionic and butyric fermentations are important in some cases.

### 3.3.1. Lactic Acid Fermentation

Lactic acid bacteria are ubiquitous. Their activities are strongly guarded against in milk for liquid consumption but are promoted in starters used in the manufacture of butter, cheese, and cultured products. Some microorganisms can produce as much as 1.5% lactic acid in milk. These bacteria are classified as homofermentative if they produce only lactic acid and heterofermentative if they produce acetic acid, alcohol, and $CO_2$ as well as lactic acid. Besides the end products of the fermentations, small quantities of intermediates, notably pyruvic acid, accumulate in the milk.

Homofermentative bacteria of the so-called group N streptococci (*S. cremoris*, *S. lactis*, and *S. diacetylactis*) have a phosphotransferase system in the cell membrane that phosphorylates lactose at C-6 of the galactose moiety as it enters the cell. A β-D-phosphogalactosidase then hydrolyses the lactose-P to glucose and galactose-6-P. The glucose is phosphorylated at C-6 and metabolized to pyruvate via the Embden–Meyerhof pathway of glycolysis:

$$\text{glucose} + \text{ATP} \xrightarrow{\text{glucokinase}} \text{glucose-6-P} + \text{ADP} \qquad (3.5)$$

$$\text{glucose-6-P} + \text{ATP} \xrightarrow{\text{2 steps}} \text{fructose-1,6-P} + \text{ADP} \qquad (3.6)$$

$$\text{fructose-1-6-P} \xrightarrow{\text{aldolase}} \text{dihydroxyacetone-P} + \text{glyceraldehyde-3-P} \qquad (3.7)$$

dihydroxyacetone-P $\xrightarrow{\text{isomerase}}$ glyceraldehyde-3-P (3.8)

2 glyceraldehyde-3-P + 2 ADP + 2 $NAD^+$ + 2 $H_3PO_4$ $\xrightarrow{\text{5 steps}}$ 2 phosphoenol pyruvate + 2 ATP + 2 NADH + 2 $H^+$ + 2 $H_2O$ (3.9)

2 phosphoenol pyruvate + 2 ADP $\xrightarrow[\text{kinase}]{\text{pyruvate}}$ 2 pyruvate + 2 ATP (3.10)

2 pyruvic acid + 2 NADH + 2 $H^+$ $\xrightarrow[\text{dehydrogenase}]{\text{lactic}}$ 2 lactate + 2 $NAD^+$ (3.11)

Net: glucose + 2 ADP + 2 $H_3PO_4$ → 2 lactate + 2 ATP + 2 $H_2O$ (3.12)

The galactose-P is converted via a fructose isomer, tagatose-P, to the triose-P stage of glycolysis and thence to pyruvate. The group N streptococci all have lactic dehydrogenase with L specificity, and they produce four molecules of L(+)-lactic acid from a molecule of lactose.

Other homofermentative bacteria, such as *S. thermophilus* and *Lactobacillus bulgaricus*, do not phosphorylate entering lactose but rather hydrolyse it with an intracellular $\beta$-galactosidase. The glucose moiety proceeds through glycolysis to form lactic acid [L(+) in some species, D(−) in others, and a mixture in still others]. These organisms lack the enzymes necessary to epimerize galactose to glucose, and galactose is not metabolized. Only two molecules of lactic acid are produced from a molecule of lactose.

Some organisms (e.g., *Pseudomonas*) oxidize glucose to gluconic acid and proceed to metabolize it to two molecules of lactic acid by the Entner–Doudoroff pathway. (See equations 3.13–3.15.) The initial oxidation at C-1 of glucose is catalyzed in some cases by a lactose dehydrogenase before lactose is hydrolyzed; in other cases, glucose is oxidized after the lactose is split.

glucose-6-P + $NAD^+$ + $H_2O$ $\xrightarrow{\text{2 steps}}$ 6-P-gluconate + NADH + $H^+$ (3.13)

6-P-gluconate $\xrightarrow[\text{dehydrase}]{\text{6-P-gluconate}}$ 2-keto-3-deoxy-6-P-gluconate + $H_2O$ (3.14)

2-keto-3-deoxy-6-P-gluconate $\xrightarrow[\text{aldolase}]{\text{KDPG}}$ pyruvate + glyceraldehyde-3-P (3.15)

In the Entner–Doudoroff pathway, carbons 1 and 4 of the hexose appear as carboxyls in the lactic acid; in Embden–Meyerhof glycolysis, the carboxyls of the lactic acid come from carbons 3 and 4 of the hexose.

Heterofermentative organisms, including some members of the genera *Lactobacillus* and *Leuconostoc*, lack an aldolase like like the one that cleaves six-carbon to two three-carbon units in the Embden–Meyerhof and Ent-

ner–Doudoroff pathways. They use a pathway known as the hexose–monophosphate shunt in which 6-phosphogluconic acid is formed from glucose, as in equations 3.5 and 3.13, and then is decarboxylated, and the resulting five-carbon unit, ribulose-5-phosphate, is split to acetyl phosphate and glyceraldehyde-3-phosphate. The former yields acetic acid and/or ethanol, and the latter yields pyruvic acid and then lactic acid. The following equations outline this pathway:

$$\text{6-P-gluconate} + \text{NAD}^+ \xrightarrow{\text{2 steps}} \text{ribulose-5-P} + \text{NADH} + \text{H}^+ + \text{CO}_2 \quad (3.16)$$

$$\text{ribulose-5-P} + \text{H}_3\text{PO}_4 \xrightarrow{\text{2 steps}} \text{acetyl-P} + \text{glyceraldehyde-3-P} + \text{H}_2\text{O} \quad (3.17)$$

$$\text{glyceraldehyde-3-P} + 2\ \text{ADP} + \text{H}_3\text{PO}_4 \xrightarrow[\text{glycolysis}]{\text{as in}} \text{lactate} + 2\ \text{ATP} + \text{H}_2\text{O} \quad (3.9\text{–}11)$$

$$\text{acetyl-P} + 2\ \text{NADH} + 2\ \text{H}^+ \xrightarrow{\text{2 steps}} \text{ethanol} + 2\ \text{NAD}^+ + \text{H}_3\text{PO}_4 \quad (3.18)$$

$$\text{Net: glucose} + \text{ADP} + \text{H}_3\text{PO}_4 \rightarrow \text{lactate} + \text{ethanol} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O} \quad (3.19)$$

Galactose can enter this pathway by epimerization of its phosphate to glucose-6-phosphate. The dehydrogenases in some organisms are coupled to $NADP^+$ rather than to $NAD^+$. Acetyl phosphate, in the presence of suitable hydrogen acceptors, also can yield acetic acid.

Important properties of lactic acid are summarized in the appendix, Table A.18.

### 3.3.2. Other Fermentations

Bacteria of the genus *Propionibacterium* ferment lactic acid to propionic acid, acetic acid, $CO_2$, and water:

$$3\ \text{CH}_3\text{—CHOH—CO}_2\text{H} \rightarrow 2\ \text{CH}_3\text{—CH}_2\text{—CO}_2\text{H} + \text{CH}_3\text{—CO}_2\text{H} + \text{CO}_2 + \text{H}_2\text{O} \quad (3.20)$$

This fermentation is prominent in certain Swiss-type cheeses. Many of these organisms can ferment lactose, but in cheese they utilize lactic acid produced by the faster-growing starter bacteria.

Butyric acid fermentation of lactic acid is characteristic of certain *Clostridium* bacteria:

$$2\ \text{CH}_3\text{—CHOH—CO}_2\text{H} \rightarrow \text{CH}_3\text{—CH}_2\text{—CH}_2\text{—CO}_2\text{H} + 2\ \text{CO}_2 + 2\ \text{H}_2 \quad (3.21)$$

This fermentation is highly undesirable in cheese because of the gas and off-flavor.

*Streptococcus thermophilus* and *Lactobacillus bulgaricus*, in addition to the homofermentative production of lactic acid mentioned in Section 3.3.1., divert some pyruvic acid to the production of diacetyl:

$$2\,CH_3—CO—CO_2H + O \rightarrow CH_3—CO—CO—CH_3 + 2\,CO_2 + H_2O \quad (3.22)$$

Certain other organisms, such as *S. diacetylactis* and *Leuconostoc cremoris*, also can oxidize pyruvic acid to diacetyl, but they have the additional ability to ferment citric acid to pyruvic acid. Diacetyl is an important flavor compound in some dairy products.

## 3.4. OTHER CARBOHYDRATES

Free glucose and galactose are detected readily in fresh milk, but there is a lack of agreement on their concentrations. Enzymatic analysis indicates concentrations of about 0.1 m*M* glucose and 0.2 m*M* galactose, and for glucose at least this seems close to its concentration in the cell. On the other hand, concentrations three to eight times higher have been found by gas–liquid chromatographic techniques. Other carbohydrates found free in solution in milk (Section 8.1) are amino sugars, sugar phosphates, neutral and acid oligosaccharides, and nucleotide sugars. In part, at least, they probably represent building blocks of other larger molecules synthesized by the mammary gland.

Some proteins are glycosylated (Section 6.2) and some lipids contain carbohydrate moieties (Section 5.2).

## *Suggested Literature*

Many facts on lactose, including crystallization, are given by

T. A. Nickerson, "Lactose," in B. H Webb, A H Johnson, and J. A Alford, eds., *Fundamentals of Dairy Chemistry*, 2nd ed , (Westport: AVI, 1974)

An interesting series on lactose crystallization is

A. van Kreveld and A S. Michaels, *J. Dairy Sci* 48 (1965) 259.

A. S. Michaels and A. van Kreveld, *Neth. Milk Dairy J.* 20 (1966):163.

A. van Kreveld, *Neth Milk Dairy J* 23 (1969).258

R. A. Visser, *Neth Milk Dairy J.* 34 (1980).255.

Lactic acid fermentation by *Streptococci* is discussed by

B. A. Law and M. E. Sharpe, in F. A. Skinner and L. B. Quesnel, *Streptococci* (New York: Academic Press, 1978).

The chemistry of lactic acid is covered in

C. H. Holten, *Lactic Acid: Properties and Chemistry of Lactic Acid and Derivatives* (Weinheim: Verlag Chemie, 1971).

# 4

# Salts

This chapter discusses substances that are or can be present in milk as ions of fairly low molecular weight (say, less than 300). Polar lipids and proteins are excluded. The former may be neglected in this context; the latter, being macroions, are a class apart. The definition is not equivalent to inorganic or mineral substances, mainly because some salts are organic. Neither is it equivalent to ionic substances, because a considerable part is present in nonionized form. Certainly, it is not equivalent to "ash," since ashing of milk causes loss of organic salts and formation of ash from nonsalt substances. (See Section 20.6.) The quantities of salts can be expressed in various ways—for instance, as moles, equivalents, or grams. In the latter case, it can be the mass of the element (e.g., P), residue (e.g., $PO_4^{3-}$), or oxide (e.g., $P_2O_5$).

The salts are important from a nutritional point of view (Section 19.3), and because they largely determine the physicochemical state of the milk serum, which in turn largely determines the conformation and stability of the proteins. Moreover, specific salts cause specific actions, such as catalysis.

## 4.1. OVERALL COMPOSITION

Table 4.1 lists the most important salts of milk; more information is in Tables 1.3, 4.2, and 4.4. The amines, minor organic acids, and phosphoric esters each consist of several different substances. (See the appendix, Tables A.2, A.9, and A.11.) Fresh milk usually contains hardly any nitrate (at most, 5 mg per kilogram and usually much less) and no nitrite. Variations in the salt composition of milk are discussed in Chapter 9.

Not all of the salts are dissolved. The casein micelles contain undissolved calcium phosphate and a little citrate; it is often called "colloidal calcium

**Table 4.1. Approximate Salt Composition of Milk**

| Cationic | mmol $kg^{-1}$ | Anionic | mmol $kg^{-1}$ |
|---|---|---|---|
| Sodium (23)[a] | 17–28 | Chloride (35 5) | 22–34 |
| Potassium (39 1) | 31–43 | Carbonate[b](60) | ~ 2 |
| Calcium (40 1) | 26–32 | Sulfate (96 1) | ~ 1 |
| Magnesium (24 3) | 4–6 | Phosphate[c](95) | 19–23 |
| Amines | ~ 1 5 | Citrate[d](189) | 7–11 |
| | | Organic acids[e] | ~ 2 |
| | | Phosphoric esters[f] | 2–4 |

*Note* Range comprising most literature data excluding colostrum, late lactation and mastitic milks Individual cows samples give wider variation
[a]Within parentheses is the atomic or residue weight
[b]Including $CO_2$
[c]Inorganic only
[d]About 1% of this is isocitrate
[e]Other than citric acid
[f]Some also contain a basic group

phosphate " (See Section 4 4 ) Some cations, notably $Ca^{2+}$ and $Mg^{2+}$, are associated with the negatively charged proteins Small quantities of other ions (e g , $Cl^-$) also may be bound to the proteins Almost all of the salt is in the serum or in the casein micelles, and very little is bound to the fat globules Table 4 2 gives the approximate distribution The salt in the casein micelles can be dissolved by lowering the pH to about 4 6 or lower, and

**Table 4.2. Distribution of Some Salts between Casein Micelles and Serum**

| Compound | mg per 100 g milk | Percentage Present in Serum | mg per 100 g Serum | mg per g Dry Casein[a] |
|---|---|---|---|---|
| Na | 48 | 95 | 49 | 0 9 |
| K | 143 | 94 | 145 | 3 3 |
| Mg | 11 | 66 | 8 | 1 5 |
| Ca | 117 | 32 | 40 | 31 0 |
| Inorganic phosphate[b] | 203 | 53 | 116 | 37 0 |
| Citrate | 175 | 92 | 173 | 5 6 |

*Note* Average from various sources
[a]A small part of this may in fact be counterions of the whey proteins
[b]As $PO_4$

also, though slowly and incompletely, by dialysis against water or a calcium-free solution.

Milk contains phosphorus in many forms. It is all present as orthophosphate, but part of it is bound to organic components, either esterified to serine and threonine residues of casein, to several smaller molecules like hexoses and glycerol, or in the phospholipids. Table 4.3 gives particulars. The sulfur content of milk is about 0.36 $g \cdot kg^{-1}$, but most of it is in the amino acid residues methionine and cysteine of the proteins. About 10% is present as inorganic sulfate.

Milk contains several trace elements; they are discussed in Section 8.1.2 and listed in Table A.1.

## 4.2. THE SALT SOLUTION: COMPOSITION

The salt composition of milk (i.e., the species of salt molecules and ions present and their concentrations) is not known precisely. This is mainly because of the fairly extensive formation of ion pairs. Some of the acids or bases in milk are not ionized fully at milk pH, such as phosphoric and carbonic acid and secondary amines. But some of the salts also may be present partly as ion pairs, often to a considerable degree. This concerns primarily binding of $Ca^{2+}$ and $Mg^{2+}$ to $citrate^{3-}$ and to a lesser extent to $HPO_4^{2-}$; but also other salts (e.g., the chlorides of Na, K, Ca, and Mg) are not completely dissociated. (See the appendix, Table A.20.)

The association describes an average state; each of the ion pairs exists only for a short time, and there is a dynamic and very rapidly attained equilibrium between the ion pairs and the free ions. Likewise, there is a dynamic equilibrium between salts in the dissolved and the undissolved states, though the equilibrium may be attained much more slowly. Undissolved salts are in the colloidal calcium phosphate of the casein micelles. (See Section 4.4.)

### 4.2.1. Ion Activity

The properties of a salt solution, such as the state of ionization of the various components, are not governed by concentrations but by activities. The general relation between the activity $a$ and the (molar) concentration $m$ of a substance X is given by

$$a_X = y_X m_X \tag{4.1}$$

where $y$ is the (molar) activity coefficient. If no other solute is present, $y \rightarrow 1$ for $m \rightarrow 0$, but for finite $m$, $y$ may be less than 1. For ionic species, the situation is more complicated; usually, activities of the ions rather than the

**Table 4.3. Approximate Distribution of Phosphorus in Milk**

| Type | mg P Per kg Milk | Percentage of P | Dialysable against Water | Dialysable at Low pH | Soluble in 12% TCA[a] | Extractable with Ethanol/Ether |
|---|---|---|---|---|---|---|
| Esterified to proteins | 216 | 22 | No | No | No | No |
| In the phospholipids | 13 | 1 | No | No | No | Yes |
| Soluble esters | 90 | 9 | Yes | Yes | Yes | Yes |
| Inorganic "colloidal" | 314 | 32 | No[b] | Yes | Yes | No |
| Inorganic dissolved | 348 | 36 | Yes | Yes | Yes | No |

*Note:* Average from various sources.
[a]Trichloroacetic acid.
[b]Partly dialysable against an excess of water.

salt (or acid or base) are used. If we assume cations B of valency $\beta$ and anions D of valency $\delta$, then

$$B_bD_d \rightleftarrows b\ B^{\beta+} + d\ D^{\delta-} \tag{4.2}$$

where $b\beta = d\delta$. Calling the salt (or acid or base) BD for short, we now define the free ion activity of the salt as

$$a_{BD} = (a_B^b \cdot a_D^d)^{1/(b+d)} = (y_B^b m_B^b \cdot y_D^d m_D^d)^{1/(b+d)} \tag{4.3}$$

Here, $m$ denotes the concentration of free ions including ion pairs.

All reaction rates of the ions depend on their activities. Hence, for the dissociation equilibrium of equation 4.2, we have a dissociation constant:

$$K_{BD} = \frac{a_B^b \cdot a_D^d}{a_{BD}^*} \tag{4.4}$$

where $a_{BD}^*$ is the activity of the ion pair (the undissociated salt). For neutral species, the activity coefficient usually is $\approx 1$, so we may put $a_{BD}^* = m_{BD}$ if BD is neutral. If $y_B$ and $y_D$ now decrease, $a_B$ and $a_D$ decrease; consequently, $m_{BD}$ must decrease to keep $K_{BD}$ constant. In other words, if the activity coefficients decrease, the dissociation increases (a smaller fraction of the substance is present as ion pairs). Often, the association constant, which is the reciprocal of the dissociation constant, is used. Examples are in the appendix, Table A.20. Usually, we only consider associations involving two ions (e.g., between $Ca^{2+}$ and $Cl^-$ giving $CaCl^+$, not between $Ca^{2+}$ and $2Cl^-$). Consequently, the exponents $b$ and $d$ in equation 4.4 would equal 1, and $K_{BD}$ is expressed in moles per liter (or kilograms).

When the activity coefficients decrease, the solubility of the salt increases. This is because a salt has a solubility product $K'$ such that the condition:

$$a_B^b \cdot a_D^d = m_B^b y_B^b \cdot m_D^d y_D^d \leq K'_{BD} \tag{4.5}$$

must be fulfilled to prevent precipitation of BD. Decrease of $y$ gives decrease of $a$, hence $m$ may become higher before the solubility product is exceeded. Note that $K'$ is expressed in (moles per liter)$^{b+d}$.

The association and dissociation constants and solubility products as used here are intrinsic constants; they pertain to activities and can apply only to concentrations at infinite dilution. Often, we use stoichiometric constants that relate to concentrations, but then the value of the constant depends on the activity coefficients and thus on conditions.

Usually, the activities are unknown because classical chemical analysis gives concentrations. Equilibrium methods, such as measurement of potentials with ion-selective electrodes, yield activities; for instance, pH $= -\log a_{H^+}$. Often, relative rather than absolute activities (or activity coefficients) are found in fact, so we should use ratios of $a^{1/z}$ or $y^{1/z}$, where $z$ is the (absolute value of the) valency. Usually, we try to find values of the activity coefficients from theory. They depend primarily on the total (effective) ionic strength $I$ given by

$$I = \tfrac{1}{2} \sum m_i \cdot z_i^2 \tag{4.6}$$

Here, $y = 1$ for $I \to 0$, but it falls rapidly with increasing $I$, the more so if $z$ is larger. A crude explanation is that the charge of each ion is screened by the other ions, the more so when their concentration is higher. Though other factors also have effect, we usually assume that $y$ depends on $I$ and $z$ only, and roughly (at room temperature)

$$y_i \approx \exp(-0.8\, z_i^2 I^{1/2}) \tag{4.7}$$

Under conditions as in milk ($I \approx 0.08$), $y$ for mono-, di-, and trivalent ions is probably about 0.8, 0.4, and 0.13, respectively. It follows that in the case of ions (unlike that of most electroneutral species), the difference between activity and concentration may be considerable. For example, the stoichiometric solubility product of $Ca_3citrate_2$ is nearly 1000 times as large in milk as it would be for $I = 0$. Values of the p$K$ ($-\log K$ = pH at which 50% is dissociated) of acids are shifted downward by 0.1–0.5 units in milk, as compared to $I = 0$.

Be cautioned that there are minor uncertainties, differences of opinion, and differences in manner of expression in the activity theory. The uncertainties are greater when multicomponent interactions are involved. Several dissociation constants are not known exactly. Sometimes, the total instead of the free ion activity coefficient is used; it relates ion activity to total salt, rather than free ion concentration, and is thus smaller than the free coefficient if ion pairs are present.

### 4.2.2. Estimating Dissolved Salts

To arrive at the concentration of the various ions and neutral salt molecules in milk, the overall concentration of dissolved salts must be known.

The salt solution of milk can be obtained by dialysis of water against a large excess of milk. Ultrafiltrates, if obtained at low pressure, have nearly the same composition as equilibrium dialysates. Serum obtained by ultracentrifugation and rennet whey differ more, because of the presence of whey proteins.

The composition of a dialysate does not reflect precisely the concentrations of the various dissolved substances in milk. First, we should consider the fact that part of the water in milk is not available as a solvent; this is largely because of the protein and is discussed further in Section 17.1.2. The amount of nonsolvent water increases with the molecular size of the solute, and it varies from about 0.15–0.60 g of nonsolvent water per gram of protein, that is, from 0.5–2.3% of the water present. (See Section 20.1.3 for equations to convert concentrations.)

Another effect comes from the electric charge of the colloidal particles (fat globules, casein micelles, and whey protein molecules, all negatively charged at milk pH). The charge causes a change in the concentration of

ions nearby. Ions of opposite charge, called counterions, accumulate near a charged surface; in milk, most counterions are cations. The excess concentration is highest at the surface of the particles and diminishes exponentially with distance. We speak of an "ion atmosphere" or "diffuse double layer"; its thickness, defined as the distance over which the surface charge has been compensated (shielded) by the counterions to $1/e$ of its value, is about 1 nm in milk (i.e., comprising perhaps 3% of the volume of milk).

Part of the ions associated with the protein will be truly bound; they have formed ion pairs with ionized groups of the proteins. Ester phosphate groups are likely binding sites for $Ca^{2+}$ ions. The bound ions neutralize part of the surface charge of the proteins. It is not possible, however, to distinguish truly between ions neutralizing (bound ions) and ions shielding the charge (ions in the double layer). For convenience, we will group them both under the heading of counterions. It is thus not known how many of the counterions are truly bound; it is often assumed that all of them are, but this is certainly not true. Usually, it is taken that all counterions are associated with the casein micelles, but about 6% are associated with the whey protein and 2% with the fat globules. The binding of the different cations, or their crowding in the double layer, is not in proportion to their activity in the solution. The larger the charge (and the smaller the diameter) of an ion, the higher its concentration in the double layer (for the same activity). Consequently, $Ca^{2+}$ and $Mg^{2+}$ will be important counterions. Of the cations associated with the protein, all the Na and K, most of the Mg, and about 20% of the Ca probably are present as counterions; the rest is in the colloidal phosphate. Some hydrogen ions also will act as counterions, and their concentration, and to a somewhat lesser extent their activity, will be higher near the protein surface; hence, the pH will be lower (perhaps by a few tenths of a unit).

A milk dialysate thus contains less counterions (i.e., cations) than milk does. By the same argument, it contains more coions (i.e., anions); electrostatic repulsion diminishes their concentration near the protein surface. Precise calculations cannot be made, but a rough estimate on, for instance, $Cl^-$ indicates an increase in concentration of at least 2% over that in milk (expressed per kilogram of water). The increase found experimentally is, at most, 1%. Presumably, a small amount of $Cl^-$ is bound to the protein, which is a common phenomenon (it would amount to $\sim 0.3$ mol $Cl^- \cdot mol^{-1}$ of protein). Nothing is known about the binding of other anions.

The salt composition of milk and that of milk dialysate are fairly well known. For some ions, activity ratios have been determined—for instance, $a_{Ca^{2+}}/(a_{K^+})^2$ and $a_{Mg^{2+}}/(a_{K^+})^2$. The hydrogen ion activity is known from the pH. The dissociation of acids depends, of course, on the pH. The relation is

$$\log\left(\frac{[\text{ion}]}{[\text{acid}]}\right) = \text{pH} - \text{p}K \tag{4.8}$$

The stoichiometric dissociation constants of the important acids are approximately, for the ionic strength of milk and expressed as p$K$s,

Phosphoric acid—2.1, 6.9, 12.0
Citric acid—3.0, 4.5, 4.9
Carbonic acid—6.3, 10.0
Most monobasic carboxylic acids—~ 4.7

Finally, the association constants of most salts are roughly known; some are tabulated in appendix Table A.20. Taking further into account that the solution should be electrically neutral, the ion composition of the milk salt solution can be calculated.

The approximate average composition is given in Table 4.4. Because of uncertainties, it cannot be entirely precise. All species present in concentrations below 0.05 m$M$ have been omitted. There is, of course, a significant variation between lots of milk. The table gives the composition of the solution, excluding all colloidal particles and macromolecules with their diffuse double layers; it is essentially the composition of milk dialysate. The activities in this solution are determinant for equilibria and reaction rates. To convert the composition to millimoles per kilogram of milk, multiply by ~ 0.904; to convert to millimoles per kilogram of water, multiply by ~ 1.045.

## 4.3. THE SALT SOLUTION: PROPERTIES

Table 4.4 gives an effective ionic strength $I$ of (73.5 + 85.1)/2 = 79 m$M$. Ionic strength is very important for the stability of colloid particles and for the conformation of macroions, such as proteins. It mainly affects the thickness of the diffuse double layer $1/\kappa$. Assuming the relative dielectric constant of the salt solution to be the same as that of water (~ 80), we have at room temperature:

$$1/\kappa \approx 0.3\, I^{-0.5} \tag{4.9}$$

where $1/\kappa$ is in nanometers and $I$ is in moles per liter. We thus have $1/\kappa \approx$ 1.1 nm. Consequently, electrostatic interactions in milk usually will be of little importance over distances greater than 2.5 nm. The concentration of divalent cations ($Ca^{2+}$ + $Mg^{2+}$) is low, only about 30% of the total Ca and Mg in solution; their joint activity usually is less than 2 m$M$ Nevertheless, their activity is of great importance. For instance, the molar concentration of $Ca^{2+}$ and $Mg^{2+}$ needed to cause flocculation of lyophobic colloid particles (Section 12.2) is roughly one-sixtieth of that for $Na^+$ and $K^+$. Ionized calcium has a large effect on the conformation and association of some proteins, particularly caseins. The divalent cation activity appears to be rather variable

**Table 4.4. Tentative Estimate of the Composition of the Salt Solution of Milk**

| Cationic | | | | | Anionic | | | | | Neutral | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | $m$ | $mz$ | $mz^2$ | $a$ | Species | $m$ | $mz$ | $mz^2$ | $a$ | Species | $m$ |
| $K^+$ | 36.4 | 36.4 | 36.4 | 29.0 | $Cl^-$ | 30.7 | 30.7 | 30.7 | 25.0 | KCl | 0.7 |
| $Na^+$ | 21.1 | 21.1 | 21.1 | 17.0 | $SO_4^{2-}$ | 0.9 | 1.8 | 3.6 | 0.4 | NaCl | 0.4 |
| $Ca^{2+}$ | 2.5 | 5.0 | 10.0 | 1.0 | $KSO_4^-$ | 0.1 | 0.1 | 0.1 | 0.1 | $CaSO_4$ | 0.1 |
| $CaCl^+$ | 0.3 | 0.3 | 0.3 | 0.2 | $Citrate^{3-}$ | 0.9 | 2.7 | 8.1 | 0.1 | $CaHPO_4$ | 0.5 |
| $CaH_2PO_4^+$ | 0.1 | 0.1 | 0.1 | 0.1 | $HCitrate^{2-}$ | 0.1 | 0.2 | 0.4 | — | $MgHPO_4$ | 0.3 |
| $Mg^{2+}$ | 1.0 | 2.0 | 4.0 | 0.4 | $CaCitrate^-$ | 6.5 | 6.5 | 6.5 | 5.2 | $KH_2PO_4$ | 0.2 |
| $MgCl^+$ | 0.1 | 0.1 | 0.1 | 0.1 | MgCitrate | 1.9 | 1.9 | 1.9 | 1.5 | $NaH_2PO_4$ | 0.1 |
| $RNH_3^+$ | 1.5 | 1.5 | 1.5 | 1.2 | RCOO | 1.9 | 1.9 | 1.9 | 1.5 | $CaROPO_3$ | 0.2 |
| $H^+$ | | | $2 \times 10^{-4}$ | | $HPO_4^{2-}$ | 3.9 | 7.8 | 15.6 | 1.6 | $CO_2$ | 0.6 |
| | | | | | $H_2PO_4^-$ | 6.4 | 6.4 | 6.4 | 5.1 | Lactose | 147.0 |
| | | | | | $KHPO_4^-$ | 0.5 | 0.5 | 0.5 | 0.4 | Urea | 5.0 |
| | | | | | $NaHPO_4^-$ | 0.4 | 0.4 | 0.4 | 0.3 | Other | 4.0 |
| | | | | | $ROPO_3^{2-}$ | 1.7 | 3.4 | 6.8 | 0.7 | | |
| | | | | | $HROPO_3^-$ | 0.6 | 0.6 | 0.6 | 0.5 | | |
| | | | | | $KROPO_3^-$ | 0.2 | 0.2 | 0.2 | 0.1 | | |
| | | | | | $HCO_3^-$ | 1.4 | 1.4 | 1.4 | 1.1 | | |
| Total | 63.0 | 66.5 | 73.5 | | | 58.1 | 66.5 | 85.1 | | | 159.1 |

*Source:* Partly after C. Holt, unpublished results.
*Note:* Including other dissolved substances, pH = 6.7; $m$ = concentration in mmol per liter solution; $z$ = [valency]; $a$ = free ion activity.

between lots of milk, perhaps by a factor of 1.5 for herd milk. Since milk is saturated with respect to calcium phosphate, $Ca^{2+}$ activity depends much on soluble phosphate content, as the product of the activities of $Ca^{2+}$ and $HPO_4^{2-}$ must be roughly constant (for a given temperature). Ionic strength varies less, perhaps by a factor of 1.2.

The electrical conductivity is largely determined by the salt solution. It is about 0.5 $A \cdot V^{-1} \cdot m^{-1}$ (variation ~ 0.40–0.55) at 25°C. This roughly corresponds with the conductivity of 0.25% (w/w) NaCl in water.

The pH of milk dialysate is a function of its salt composition and temperature. The presence of the proteins in milk does affect this pH, of course, as it affects the salt composition. The titratable acidity of milk depends both on its salt composition (largely phosphate content) and its protein content. (See Section 11.3.)

The freezing point of milk follows from the total concentration of dissolved substances. These are given in Table 4.4. The freezing point depression of water is 1.86 K times the osmotic coefficient times the concentration in mole solute per kilogram of water. For the ionic species, the average osmotic coefficient is ~ 0.93; for the nonionic species, it is ~ 1.00. Taking into account the factor 1.045 to convert to moles per kilogram of water, we calculate from Table 4.4 a freezing point depression of 0.53 K. The determined freezing point of milk is −0.53–−0.57°C, but the commonly used method (Hortvet) has a systematic error, giving a freezing point 0.017 K too low. The freezing point corresponds to that of a solution of about 0.85% (w/w) of NaCl. It follows from Table 4.4 that lactose accounts for ~ 54%, and Na, K, and Cl for ~ 31% of the freezing point depression.

The freezing point depression of milk is very constant, since it is proportional to its osmotic pressure, which is essentially equal to that of blood, which in turn is kept almost constant. It is about 700 kPa (≈ 7 atm) at 20°C. The other colligative properties of milk are boiling point elevation of ~ 0.15 K, and water activity of ~ 0.993.

## 4.4. THE COLLOIDAL CALCIUM PHOSPHATE

Milk always is saturated with respect to calcium phosphate, a large part of which is indeed undissolved. For instance, the stoichiometric solubility product of $CaHPO_4 \cdot 2H_2O$ at the ionic strength of milk is ~ $16 \times 10^{-7}$. If all calcium phosphate were dissolved, the hypothetical concentration product would be some $2 \times 10^{-4}$; the actual one is about $10^{-5}$. (See Table 4.4.) Consequently, milk is probably supersaturated with respect to dicalcium phosphate. It appears to be even more supersaturated with respect to $Ca_4H(PO_4)_3$ (octacalciumphosphate) and $Ca_5OH(PO_4)_3$ (hydroxyapatite). Apparently, thermodynamic equilibrium is not attained, though there may be uncertainties in the solubility products. Probably, most lots of milk are saturated with respect to $Ca_3$citrate$_2$; its stoichiometric solubility product at

$I = 0.08$ is about $2 \times 10^{-15}$. The concentration products of $MgHPO_4$, $CaCO_3$, $MgCO_3$, and $Mg_3citrate_2$ are well below saturation. Some solubility products are listed in the Appendix, Table A.19.

As shown in Section 4.2, milk dialysate contains much less Ca and inorganic phosphate than milk does, and the rest is largely associated with the casein micelles. Expressed per mole of casein, there is typically about 18.0 mol Ca, 1.4 mol Mg, 2.0 mol K, 0.9 mol Na, 9.1 mol phosphate, and 0.7 mol citrate associated with the casein micelles. Part of this is an undissolved salt mixture, usually called colloidal calcium phosphate. It is difficult to decide, however, how much of the inorganic material is indeed undissolved salt. Part of the cationic material is bound to or present as counterions to the casein, presumably about 4 mol $Ca^{2+}$, almost all of the $Na^+$ and $K^+$, and most or all of the $Mg^{2+}$. Other substances also may be bound directly to the casein, perhaps 0.1 mol citrate and 0.3 mol Cl per mole of casein. Other complications are that part of the counterions (~ 8%) will not be associated with casein but with whey proteins and fat globules, and that the exclusion of coions may lead to a slight underestimation of the phosphate and citrate contents of the casein micelles.

The composition of the colloidal calcium phosphate has been the subject of considerable debate. The different types of Ca (associated with casein and inorganic phosphate, respectively) cannot be separated. It is even uncertain in principle whether they can be distinguished (i.e., whether it can be stated rightly that a certain Ca ion is bound to casein or to inorganic phosphate). Despite these experimental and conceptual uncertainties, it is clear that the molar ratio Ca/P (Ca bound to inorganic phosphate/inorganic phosphate) is fairly high, say ~ 1.5–1.6. Taking the other ions into account, we may have (Ca + Mg)/(P + citrate) ≈ 1.5. These ratios vary between lots of milk. Comparing the pH of milk (~ 6.7) with the $pK_2$ of phosphoric acid (~ 6.9), we would rather expect $CaHPO_4$ to precipitate, hence Ca/P = 1. Several substances (e.g., $Mg^{2+}$, citrate, and various proteins) seem to stabilize amorphous calcium phosphates of higher Ca/P ratios. This matter is very complicated, since calcium phosphates can exist in many compositions and forms, both amorphous and crystalline, and thermodynamic equilibrium is attained very slowly, if at all. (The absence of a thermodynamic equilibrium state for the colloidal calcium phosphate does not detract from the fact that it is in dynamic equilibrium with the dissolved molecules or ions.) The possible presence of Mg and citrate in the colloidal phosphate is remarkable, since their concentrations (or rather activities) in solution do not exceed solubility. But an amorphous calcium phosphate, precipitating from a solution that contains citrate, also incorporates some citrate. Consequently, the colloidal calcium phosphate of the micelles may accommodate other ions to some extent.

It makes little sense to assign a definite stoichiometry or even a crystalline structure to the undissolved calcium phosphate. If it would crystallize, it probably would be as hydroxyapatite (Ca/P ideally 1.67, but variable), but

the presence of Mg, phosphate esters, and some proteins may retard or even prevent such a transformation The presence of the casein is probably of paramount importance Since the calcium phosphate is present throughout the casein micelles (see Section 13 1), any regions of calcium phosphate must be very small, containing 15 molecules or less A crystal structure cannot be assigned to such small regions, and no or very little structure has been perceived by X-ray diffraction Moreover, such small separate deposits always would be subject to aging, to minimize their surface free energy This implies that some of the regions of calcium phosphate would grow at the expense of the other (smaller) ones (i e , they would exhibit Ostwald ripening, discussed in Section 12 1 3) Since the aging does not happen, there must be bonds between casein and calcium phosphate so that the bond free energy can compensate for the surface free energy The free $—NH_3^+$ groups of the casein are linked to some extent to the inorganic phosphate The ester phosphate groups of the casein may also play a part, since the number and location of these groups affect the interaction with calcium phosphate

The quantity of inorganic matter in the micelles varies considerably between lots of milk (e g , from 6–10 g per 100 g casein) The colloidal calcium phosphate must be the principal variable An important cause is probably variation in citrate content of the milk, since citrate strongly binds and solubilizes Ca (See Table 4 4 )

## 4.5. CHANGES IN SALTS

Because of the numerous dynamic equilibria between the salts in milk, changes in composition or conditions have many effects The proteins participate in these changes, since the ionization of several side groups, the exposure of side groups, the specific binding of some small molecules, and the ion atmosphere may all change with conditions, and subsequently influence the salt solution Since milk contains undissolved calcium phosphate, it has a buffering capacity for some ions, notably $Ca^{2+}$ Altering milk (e g , temperature, pH, adding substances) thus has a different effect on the salt solution from that of imposing the same changes onto milk serum or dialysate In milk, it may take a long time to establish equilibrium after conditions have been changed, mainly because of the precipitation or dissolution of colloidal calcium phosphate Table 4 5 gives some approximate examples of what will happen when small amounts of substances are added to milk Large additions cause greater changes, and since ionic strength increases, ion activity coefficients decrease, extrapolation of the changes given in Table 4 5 to large additions is not justified

During holding of milk, some changes may occur It loses $CO_2$; the original content in the udder is roughly twice that mentioned in Table 4 1 Enzyme action may liberate phosphoric acid from its esters, this causes an increase in the amount of colloidal phosphate and a decrease in pH (as shown in

**Table 4.5. The Effect of Various Additions to Milk on Increase (+) or Decrease (−) of Ca and Inorganic Phosphate**

| Substance Added | Effect on the Concentration[a] Of | | |
|---|---|---|---|
| | $Ca^{2+}$ | Dissolved Ca | Undissolved Phosphate |
| HCl | + 0.2 | + 0.3 | − 0.2 |
| NaOH | − 0.1 | − | + 0.2 |
| NaCl | + | + 0.006 | − |
| $CaCl_2$[b] | + 0.3 | + 0.3 | + 0.4 |
| Citric acid[b] | − 0.1 | + 0.4 | − 0.2 |
| Phosphoric acid[b] | − 0.05 | − 0.1 | + 0.1 |
| EDTA[b,c] | − | + | − |

[a]As far as is known, the approximate magnitude of the change is given in mole per mole added, for a small addition. After various sources.
[b]Plus as much NaOH as is needed to keep the pH constant.
[c]EDTA = ethylene diamine tetraacetic acid, a chelating (sequestering) agent.

equation 4.10, later on). The amount and composition of colloidal calcium phosphate may also change on storage even if conditions remain constant, but this has not been well studied. Lipolysis produces free fatty acids; they decrease the pH and bind some $Ca^{2+}$. In cream, the effects of lipolysis may be considerable. The calcium salts of fatty acids are poorly soluble; the solubility product of $Ca(RCOO)_2 \approx 10^{-10} - 10^{-12}$ $(mol^3 \cdot kg^{-3})$.

The acidity may change with additions, or by concentrating or heating the milk. Most striking is the lowering of pH by bacterial fermentation of lactose to (mainly) lactic acid. The principal effect is the dissolution of colloidal phosphate and the decrease in association of cations with the protein, the latter because the negative charge of the proteins diminishes. Some trends are illustrated in Figure 4.1. Moreover, the dissociation of most acids diminishes (phosphoric, citric, lactic, carbonic, and fatty acids). The ionic strength and $Ca^{2+}$ activity increase. The freezing point decreases by about 2 mK per millimolar increase in lactic acid concentration; the electric conductivity increases by about 4 $mA \cdot V^{-1} \cdot m^{-1} \cdot mmol^{-1}$. Several lactic acid bacteria also break down citrate, and this may enhance the increase in $Ca^{2+}$ activity. Changes caused by lactic fermentation are thus not precisely equal to those caused by adding, say, HCl to the same pH.

Changes occurring on increase of pH (e.g., by adding NaOH) are less well studied. The amount of undissolved calcium phosphate increases, and it also seems to change in composition (higher Ca/P ratio). $Ca^{2+}$ activity decreases; hence, the quantity of $Ca^{2+}$ bound or present as counterions also may decrease.

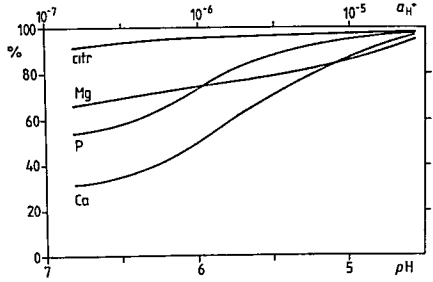



**Figure 4.1.** Effect of the acidity of milk (changed by adding HCl) on the percentages of Ca, inorganic phosphorus, Mg, and citrate that are not associated with the casein micelles. After D. T. Davies and J. C. D. White, *J. Dairy Res.* 27 (1960):171.

Temperature has many effects. We should distinguish among three types of experiments:

1. Measure properties of milk at various temperatures. Very few such measurements have been made. For instance, the pH measured at ~ 95°C is ~ 1 unit lower than at 20°C, but the reliability of such measurements is doubtful. We may try to infer the composition of the salt solution at different temperatures from the following experiment.
2. Separate milk serum or dialysate at various temperatures and investigate it at room temperature. It then follows that the pH of dialysate made at 95°C is ~ 0.5 unit lower, and that made at 0°C is ~ 0.1 unit higher, as compared to dialysate made at 20°C. (See also Figure 11.4.)
3. Heat milk at various temperatures for different times, cool to room temperature, and investigate it. This has been done often; it gives no indication of rapidly reversible changes.

Increase in temperature diminishes $CO_2$ concentration. The most important change is that calcium phosphate becomes insoluble. The reaction is roughly

$$3\ Ca^{2+} + 2\ HPO_4^{2-} \rightarrow Ca_3(PO_4)_2 \downarrow + 2\ H^+ \qquad (4.10)$$

and more $H^+$ ions will be liberated by converting $H_2PO_4^-$ to $HPO_4^{2-}$. This implies a drop in pH, probably by about 0.1 unit per millimolar decrease in dissolved phosphate. The phosphate becoming insoluble largely associates with the casein micelles. If milk dialysate is heated, amorphous calcium phosphate precipitates. During heating of whey, part of the calcium phosphate becomes associated with the whey proteins. The reactions are slow;

they reverse at room temperature, though very slowly. Figure 4.2 gives examples for Ca in milk; results for phosphate are similar. At low temperature, the reverse occurs; after 24 h at 3°C, dialysable Ca is increased by about 7%, dialysable (inorganic) phosphate is increased by 4%, and $Ca^{2+}$ concentration is increased by 13%. The magnitude of these changes is rather variable.

Upon heating at high temperatures (greater than 100°C), phosphoric acid is hydrolyzed from casein, organic acids are formed from lactose, and amino groups are lost by Maillard reactions. Hence, the pH of the milk (at 20°C) drops further, by nearly a unit when heating is severe. (See also Section 10.1.)

Concentration of the milk by evaporating water causes many changes, but heating is usually involved as well. Most dissolved substances increase about proportionally to the concentration factor, but $Ca^{2+}$ concentration increases less (e.g., by ~ 50% for a 2:1 concentration) and the quantity of colloidal calcium phosphate increases. The ionic strength increases, but less than proportionally to concentration, because ionization of several salts and acids decreases. Nevertheless, ion activity coefficients decrease. $Ca^{2+}$ activity may increase by, say, 30% on 2:1 concentration. The fraction of Ca and phosphate that is dialysable decreases only a little; it may well decrease further when holding the concentrated milk; but the pH drops on concentration, by about 0.3 units for 2:1 and about 0.5 units for 3:1 concentration. Concentrating by membrane processes is discussed in Section 17.5.

In electrodialysis, low molecular weight ions can be removed selectively. The actual selectivity of the membranes varies between 0.80–0.98 (1 minus selectivity equals flux of ions to be removed/flux of other ions). Moreover, the rate of removal of different ions greatly depends on the size of the hydrated ion; the order is, for instance, $Cl^- > K^+ > Na^+$. Ions also can be removed by passing milk over an ion-exchange column.

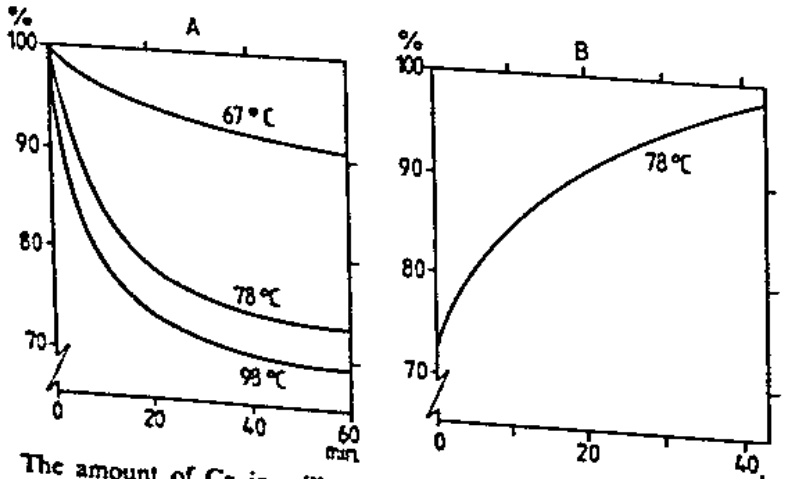



**Figure 4.2.** The amount of Ca in milk that is not associated with the casein micelles in percentage of the original amount. (A) Effect of heating at various temperatures for various times (determined after ~ 1 h at 20°C); (B) effect of holding heated milk (30 min) at 20°C. After R Jenness and S Patton, *Principles of Dairy Chemistry* (New York. Wiley, 1959, ch. 11).

## *Suggested Literature*

A comprehensive and rigorous treatment of salt solutions is given in

R. M. Pytkowicz, ed., *Activity Coefficients in Electrolyte Solutions*, vols. 1 and 2 (Boca Raton: CRC Press, 1979).

Salt composition of milk is treated by

J. C. D. White and D. T. Davies, *J. Dairy Res.* **25** (1958): 236–55.

A review of milk salts and their effects on milk properties is

G. T. Pyne, *J. Dairy Res.* **29** (1962): 101–30.

The distribution of salts between casein micelles and dialysate is treated by

D. T. Davies and J. C. D. White, *J. Dairy Res.* **27** (1960): 171–90.

A review of amorphous calcium phosphate is by

A. A. Posner and F. Betts, *Acc. Chem. Res.* **8** (1975): 273–81.

# 5

# Lipids

Lipids are esters of fatty acids and similar or derived components that are soluble in nonpolar organic solvents and insoluble, or nearly so, in aqueous liquids. The terms *lipid* and *fat* are used more or less interchangeably, though a fat usually is considered to consist largely of a mixture of neutral triglycerides, while a lipid need not be. The term *oil* is mostly reserved for fats that are fully liquid at room temperature, while a fat may be partially solid.

The fat content of milk usually is defined as the amount found by the standard Röse–Gottlieb method. This is an arbitrary definition. A small part of the phospholipids and most of the free fatty acids are not recovered by this method.

Milk fat provides the consumer with energy (~ 37 $kJ \cdot g^{-1}$) and nutrients (essential fatty acids, vitamins). The greater part of it can be separated easily from milk, which is one of the reasons why it has received so much study. In earlier times, butter was by far the most valuable product derived from milk. Products rich in milk fat, like cream and butter, have a specific and often desired texture and flavor. On the other hand, milk fat is prone to deterioration leading to serious off-flavors; such problems usually increase with the fat content of the product.

## 5.1. COMPOSITION

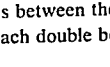
Table 5.1 gives the main lipid classes of milk fat with some of their properties. Note that about 98% of milk fat is a mixture of triacyl glycerides. There are numerous neutral lipids in trace amounts that are not on the list, such as fat-soluble vitamins and pigments. (See the appendix, Table A.4.)

The chemical and physical properties of a lipid depend primarily on chemical class; triglycerides are different from lecithins or cholesterol. But each

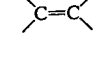
lipid class, again, consists of many different kinds of molecules with varying component fatty acid residues. This fatty acid pattern is an important factor in determining lipid properties, such as melting range (Section 5.7), chemical reactivity (Section 5.6), and nutritional value (Section 19.4). Moreover, fatty acid pattern is highly variable, whereas the relative quantities of the various classes are far more consistent.

The main variables are the following:

1. *Chain Length.* Most fatty acids contain 4–18 carbon atoms; even-numbered acids are predominant, but odd-numbered ones occur too. A common notation is $C_{18}$ for fatty acids with 18 carbon atoms.
2. *Number of Double Bonds.* Saturated fatty acids have no double bonds (e.g., stearic acid $C_{18\,0}$); unsaturated ones do. $C_{18\,2}$ thus means a fatty acid with 18 carbon atoms and two double bonds. Fatty acids with one double bond are called monoenoic, those with two are dienoic, and so on. The double bonds in the carbon chain make the fatty acid unsaturated; 2 H can be added to it per double bond at high temperature with a suitable catalyst. The reactivity of the fatty acids generally increases with the degree of unsaturation.
3. *Position of Double Bonds.* Many positional isomers can occur. Therefore, they have to be specified. $C_{18\,2}$ $\Delta 9,12$, for instance, indicates that the double bonds occur at the ninth and twelfth bonds, counting from the carboxyl group. At high temperatures (e.g., 150°C), the position of the double bonds may change. When two or more double bonds occur in a carbon chain, it is customary to distinguish between conjugated (—CH=CH—CH=CH—) and the more common nonconjugated bonds (e.g., —CH=CH—$CH_2$—CH=CH—, with one or more $CH_2$ groups between the double bonds).
4. *Geometric Isomers.* Each double bond can be either in the *cis*



or in the *trans* position



The *cis* form is the common one in nature. Linoleic acid, for example, is *cis,cis* $C_{18\,2}$ $\Delta 9,12$. But in some literature, particularly on oil processing, linoleic acid is meant to include all $C_{18\,2}$ acids. The same

**Table 5.1. The Lipids of Fresh Milk: An Overview**

| Lipid Class | Alcohol Residue | Other Constituent | MW | Component Fatty Acids | | | Percentage in milk fat (w/w) | Percentage of the Lipid In | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number | r | y | | Core of Globule | Globule Membrane | Milk Plasma |
| Neutral glycerides | | | | | | | 98.7 | | | |
| Triglycerides | Glycerol | | 728 | 3 | 14.4 | 0.35 | 98.3 | ~100 | + | + |
| Diglycerides | Glycerol | | 536 | 2 | 14.9 | 0.38 | 0.3 | 90? | 10? | ? |
| Monoglycerides | Glycerol | | 314 | 1 | 15.0 | 0.36 | 0.03 | + | + | + |
| Free fatty acids | — | | 253 | | 15.8 | 0.36 | 0.1 | 60 | 10? | 30 |
| Phospholipids[a] | | Phospho group | | | | | 0.8 | – | 65 | 35 |
| Lecithin | Glycerol | Choline | 764 | 2 | 17.2 | 0.60 | 0.26 | | | |
| Ph. ethanolamine[b] | Glycerol | Ethanolamine | 742 | 2 | 17.9 | 1.00 | 0.28 | | | |
| Ph. serine[b] | Glycerol | Serine | 784 | 2 | 17.8 | 0.80 | 0.03 | | | |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Phosphatidic acid | Glycerol | Phosphate | 855 | 2 | | | 0 04 | | | |
| Phosphatidylinositides[c] | Glycerol | Inositol | ~700 | 1[f] | | | 0 02 | | | |
| Plasmalogens | Glycerol | Choline[e] | 770 | 1 | 19 0 | 0 20 | 0 16 | | | |
| Sphingomyelin[d] | Sphingosine | Choline | 770 | 1 | 20 0 | 0 20 | 0 1 | – | 70 | 30 |
| Cerebrosides[c,d] | Sphingosine | Hexose | ~1600 | 1 | | | 0 01 | – | 70? | 30? |
| Gangliosides[c,d] | Sphingosine | Hexose[g] | | | | | 0 32 | 80 | 10 | 10 |
| Sterols | | | | | | | 0 30 | | | |
| Cholesterol | | | 387 | | | | 0 02? | | | |
| Cholesteryl esters | Cholesterol | | 642 | 1 | 16 0 | 0 40 | | | | |
| Carotenoids + vitamin A | — | | | | | | 0 002 | 95? | 5? | + |

*Note* Not complete, approximate average values from various sources $x$ = number of carbon atoms, $y$ = number of double bonds

[a] A small fraction (e g , 1%) is present as lysophosphatides

[b] Phosphatidyl ethanolamine + serine = cephalin

[c] Glycolipids

[d] Sphingolipids

[e] Or ethanolamine

[f] Also a fatty aldehyde residue

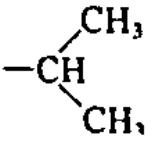
[g] Also neuraminic acid



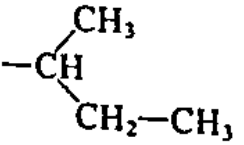
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confusion applies to oleic acid and $C_{18:1}$, or linolenic acid and $C_{18:3}$. Again, at high temperatures, *cis* may change into *trans*.

5. *Branching*. Until now, we have tacitly assumed the carbon chain to be unbranched, hence $C_{18:2}$ meant *n* $C_{18:2}$. But branched fatty acids also occur. These may be either *iso* acids, having the terminal group

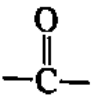


or *anteiso* with

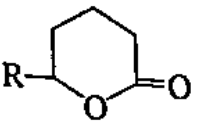


There are also traces of multibranched and cyclic fatty acids.

6. *Keto and Hydroxy Groups*. The keto or oxo fatty acids may have the



group at various positions; the 3-ketoacid residues give rise to free methyl ketones ($R–CO–CH_3$) on heating. Hydroxy fatty acids also have the –CHOH– group at various positions; some are lactonegenic, that is, are 4- and 5-hydroxy fatty acid residues that give γ- and δ-lactones on heating. These compounds have a distinct flavor. Traces of δ-lactones



are present in fresh milk fat and are essential to its flavor, but higher quantities, which may arise from heating or during long storage of dried milk or butter oil, cause an atypical flavor.

7. *1-Glycerol Ethers*. On hydrolysis, the glycerides may yield fatty alcohols and thus contain the group $–C–O–CH_2–R$; most of these come from the phospholipids. But hydrolysis may also yield fatty aldehydes. The lipids are then called plasmalogens (either neutral or

phospholipid), and they contain the group –C–O–CH=CH–R (enol isomer), yielding by hydrolysis HO–CH=CH–R → O=CH–$CH_2$–R.

Table 5.2 gives data on the fatty acid pattern. It is clear that the pattern is quite different between lipid classes. (See also Table 5.1.) Note that Table 5.2 gives the proportions in mole percentage. This facilitates calculations. Most literature data are given in mass percentage, and nutritionists in general use these. (See Table 19.2.) The appendix gives more information on minor fatty acids. (See Table A.3.) The total content of essential fatty acid residues (linoleic, linolenic, and arachidonic) is usually 2.4–3.0% (w/w).

The composition of milk fat, however, is very variable. By far the most important cause for variation is feed, and both lipid and nonlipid components affect milk fat composition. (See Section 2.3.) Consequently, it will vary with time of the year, region, farming practice, and so on. Therefore, it makes little sense to give information on the variations, unless we give a detailed account of the effect of several factors. Table 5.2 gives some idea about the range of values usually encountered in mixed milk. But if feeding is exceptional, say by feeding "protected fats" in the diet, large discrepancies may occur.

Nevertheless, the following general conclusions can be drawn about the fatty acid pattern of milk fat:

1. It contains a relatively high proportion (15–20 mol %) of short-chain fatty acid residues, $C_{4:0}$–$C_{10:0}$. Butyric acid ($C_{4:0}$) is specific for milk fat of ruminant species.
2. The proportion of saturated fatty acid residues is high; for example, 70 mol % (≈ 63% w/w).
3. Oleic acid is the most abundant of the unsaturated fatty acid residues (e.g., 70%).
4. The other unsaturated fatty acid residues are present in a wide variety of chain length, unsaturation, and isomers. The total proportion of *trans* isomers may be 5 mol % (percentage of total fatty acids).
5. There are several "odd" types of fatty acids (uneven, branched, keto, hydroxy). This makes a very great range of some 250 different fatty acid residues; to this must be added fatty alcohol and aldehyde residues.

It has been common practice to characterize the fatty acid pattern of a fat by some "fat constants," like iodine value, Reichert Meissl Wollny number, and saponification value. They are not used much any more. A list is in the appendix, Table A.5.

The variability in proportion of lipid classes is not well known, but it is probably not large. However, changes may occur, particularly as a result of lipolysis (Section 5.5), and also from oxidation (Section 5.6) and heating.

**Table 5.2. Outline of Fatty Acid Composition of Milk Lipids**

| Acid | Notation | Melting Point (°C) | Solubility at 25°C (mg/100 ml water) | Composition (in mol %) of | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Neutral Glycerides[a] | | Phospho-lipids | Free Fatty Acids[b] | Cholesteryl Esters |
| Saturated | | | | 69.0 | (57–80) | 45.0 | 72.0 | 63 |
| Butyric | 4:0 | −8 | ∞ | 8.5 | (7–14) | — | 14.5 | — |
| Caproic | 6.0 | −4 | 970.00 | 4.0 | (2–7) | — | 4.5 | — |
| Caprylic | 8:0 | 16 | 73.00 | 1.8 | (1–3.5) | 0.2 | 2.0 | 1 |
| Capric | 10:0 | 31 | 6.00 | 3.0 | (1.5–5) | 0.2 | 2.0 | 3 |
| Lauric | 12:0 | 44 | 0.55 | 3.6 | (2.5–7) | 0.5 | 2.0 | 4 |
| Myristic | 14:0 | 54 | 0.18 | 10.5 | (8–15) | 3.0 | 9.0 | 15 |
| Palmitic | 16:0 | 63 | 0.08 | 23.5 | (20–32) | 19.0 | 21.0 | 25 |
| Stearic | 18:0 | 70 | 0.04 | 10.0 | (6–13) | 12.0 | 13.0 | 12 |
| Odd-numbered | | | | 2.5 | (1.5–3.5) | 4.5 | 2.5 | 3 |
| Branched | | | | 1.1 | (0.7–1.8) | 0.7 | 1.0 | ? |
| Other | | | | 0.7 | (0.3–2) | 5.0 | ? | ? |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | 27 0 (18–36) | 41 0 | 23 0 | 30 |
| Monoene | | | | 1 4 | ? | ? | ? |
| Palmitoleic | 16 1 Δ9[c] | | | 21 0 (13–28) | 38 0 | 19 8 | 24 |
| Oleic | 18 1 Δ9[c] | 16 | | 5 5 | 3 0 | 3 5 | 6 |
| Other (unspecified) | | | | 2 5 (1–4 3) | 8 0 | | |
| Diene | | | | 1 8 | 8 0 | 2 3 | 2 |
| Linoleic | 18 2 Δ9,12[c] | −5 | 21 00 | 0 7 | 0 2 | ? | |
| Other | | | | 0 8 (0 4–2) | 4 0 | | |
| Polyene | | | | 0 4 | 2 0 | ? | |
| α-linolenic | 18 3 Δ9,12,15[c] | −12 | | 0 4 | 2 0 | ? | |
| Other | | | | 0 3 | ? | ? | |
| Keto | | | | 0 3 | ? | ? | |
| Hydroxy | | | | 0 01 | 0 15 | | |
| Fatty alcohol | | | | 0 02 | 0 01 | | |
| Fatty aldehyde | | | | | 2 0 | 1 0 | 5 |
| Unclassified | | | | | | | |

[a]In parentheses is the approximate range found in the literature
[b]The data on free fatty acids are very tentative and refer to acids liberated by the action of milk lipase only
[c]All *cis*

solvent for many other apolar substances. A small amount of water dissolves in liquid triglycerides (about 0.15% at room temperature in liquid milk fat). But triglycerides do not dissolve in aqueous media at all. However, they may be part of some lipoproteins, a kind of mixed micelles largely consisting of protein and compound lipids, and thus be present in trace quantities in milk plasma.

Physical properties are given in Sections 5.4 and 5.7 and in the appendix, Table A.21.

### 5.2.2. Di- and Monoglycerides and Free Fatty Acids

Even directly after milking, milk contains some of these substances. Table 5.1 refers to that situation. The quantity of free fatty acids shown corresponds to about 0.15 millimole per liter of milk, or about 0.4 millimole per 100 g of fat. Values reported for fresh milk range from 0.2–1.0 mmol per 100 g. Milk fat, however, is subject to lipolysis (i.e., enzymatic hydrolysis of neutral glycerides), which increases the quantities of the substances considered here, and it is often uncertain to what degree this has already occurred in apparently fresh milk. On the other hand, the composition of the free fatty acids in fresh milk markedly differs from that of the esterified ones (see Table 5.1); free butyric acid appears to be absent. This points to fatty acids of strictly fresh milk as not being formed by hydrolysis.

The enzyme causing lipolysis (milk lipase) preferentially attacks the fatty acid residues in the 1- and 3-positions. This implies that 1,2- and 2,3-diglycerides are formed, and the liberated fatty acids show a pattern different from that of milk fat as a whole. (See Table 5.2.) The diglycerides show some racemization (i.e., change of the position of the fatty acid residues) on standing. They are also subject to lipolysis, so that monoglycerides are formed, and more than can be expected from a random attack on the fatty acid residues; they are predominantly 2-monoglycerides.

Diglycerides do not differ much from the triglycerides in properties, except for the free hydroxyl group. Monoglycerides are much more polar and they are surface active; they tend to accumulate at an oil–water or an air–water interface (but not at an air–oil interface). The smaller molecules are somewhat soluble in water. In the fat phase, they probably form micelles that also contain some water.

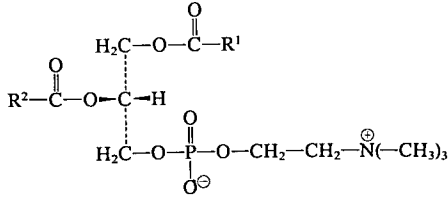
Fatty acids are also surface active and somewhat soluble in water; Table 5.2 gives the solubility in pure water. They dissociate, however, and the dissociation constants vary from $1.2 \times 10^{-5}$ to $1.5 \times 10^{-5}$, so the $pK$ is about 4.9 (i.e., at pH 4.9, 50% is dissociated). In milk plasma, they are thus predominantly in the dissociated form (i.e., as soaps), and these are much more soluble. Most sodium soaps, for instance, have solubilities that are 100 to 1000 times those of Table 5.2. Consequently, solubility in milk plasma depends much on pH. Fatty acids, but not their soaps, dissolve well in fat; they tend to associate into dimers by hydrogen bonding. There is, of course, a partition equilibrium between fatty acids in the fat and plasma phases. The

shorter the chain length, the more of the acid is in the plasma. With decreasing pH, more goes into the fat phase. The influence of pH on partitioning is largest for the short-chain fatty acids.

Besides the fatty acids derived from lipids, formic, acetic, and propionic acids are present in milk plasma. These acids and butyric acid also may be formed by microbial action from nonfat materials. Formic acid also is formed from lactose on heating.

### 5.2.3. Compound Lipids

The most abundant and best-known compound lipids in milk are the phospholipids or phosphatides, of which the phosphoglycerides make up the major portion. The formula for lecithin or phosphatidylcholine is, for example,

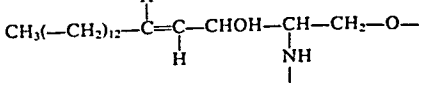


In other phosphoglycerides, the choline residue is replaced by ethanolamine ($-CH_2-CH_2-NH_3^+$), serine, or inositol (a cyclitol).

The two charged groups in the molecule give it a highly polar part. The rest is very apolar, so that the molecule is amphiphilic and does not dissolve well in either water or fat. The phospholipids, however, form micelles in water and in fat, with the polar ends at the outside and inside, respectively. They are difficult to transform from the hydrated into the other form, and vice versa. Their amphiphilic nature makes the phospholipids very surface active. In milk, they are in contact with water and with proteins as "lipoproteins." Little is known about the nature of the bonds in such complexes.

Note also, from Tables 5.1 and 5.2, that the phospholipids, particularly the cephalins, have many unsaturated fatty acid residues compared to the neutral glycerides. They include polyenoic fatty acids. This is important for lipid autoxidation.

In addition to phosphoglycerides, milk contains sphingolipids. Their common residue is sphingosine:

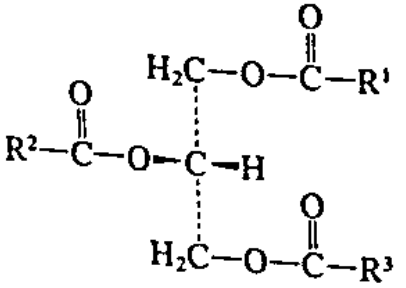


## 5.2. PROPERTIES OF VARIOUS LIPIDS

Table 5.1 gives an overview of the main classes of lipids in milk. A common division is into neutral and compound lipids, where the latter also contain appreciable nonlipid building blocks, like phospho groups, organic bases, and glucides. Another division is into saponifiable and unsaponifiable lipids. The latter do not yield fatty acids, alcohols, or aldehydes on hydrolysis. The table also gives some idea about the location of the various lipids. About 0.5% of the total fat is in the milk plasma, largely in lipoprotein particles. These exist in a wide variety. (See Section 2.5.) Only some of the short-chain fatty acids are truly dissolved in the plasma. About 1% of milk lipids is in the fat globule membrane, but the division is uncertain. (See Section 5.3.)

### 5.2.1. Neutral Triglycerides

These make up the bulk (mostly greater than 98%) of the milk fat, and thus largely determine its properties. The properties derive largely from the fatty acid pattern, but not entirely, since in mixed glycerides the distribution of fatty acids over the three positions of the glycerol residues may differ, and this yields different molecules. Consider a triglyceride mixture, containing the residues oleic (O) and stearic acid (S). It may be compounded of SSS (tristearate)and OOO. But SSO, SOS, SOO, and OSO may be present, too. As soon as a third residue occurs (e.g., palmitic acid, P) the situation is even more complicated. SSO equals OSS, but PSO and OSP are isomers, since the middle carbon atom of the glycerol residue is asymmetric:



It is common to use the *sn* (stereospecific numbering) notation. When, as in the drawing above, the C–O and C–H bonds of the middle C atom are inclined toward and both C–C bonds away from the reader, residues are numbered as shown. Now, $n$ different fatty acid residues can give rise to $n^3$ different triglyceride molecules.

Table 5.3 gives an approximate average distribution of fatty acid residues or groups of residues over the three positions. It is clear that the distribution is not random. Particularly, the preference of $C_{4:0}$ and $C_{6:0}$ for the 3-position is striking. The frequency or even the existence of specific triglycerides is

**Table 5.3. Average Arrangement of Fatty Acid Residues in Triglycerides**

| Notation | 1-Position | 2-Position | 3-Position |
|---|---|---|---|
| 4:0 | 2 | 1 | 97 |
| 6:0 | 4 | 12 | 84 |
| 8:0 | 13 | 42 | 45 |
| 10:0 | 17 | 50 | 33 |
| 12:0 | 24 | 50 | 26 |
| 14:0 | 27 | 56 | 17 |
| 14:1 | 10 | 45 | 45 |
| 15:0 | 40 | 53 | 7 |
| 16:0 | 46 | 42 | 12 |
| 16:1 | 40 | 37 | 23 |
| 18:0 | 58 | 20 | 22 |
| 18:1 | 43 | 25 | 32 |
| 18:2 | 40 | 40 | 20 |
| 18:3 | 39 | 32 | 29 |

*Note:* Percentage of each acid in designated position of *sn*-glycerol; approximate average values from various sources.

not known. The major fatty acid residues (i.e., greater than 1 mol %) would give 1331 different triglycerides. Assuming that any other, minor, residue would not appear more than once in one triglyceride molecule, we come to the order of 100,000 molecular species. Clearly, milk fat shows a wide compositional range.

Moreover, there is probably no single triglyceride present in a large quantity. Assuming the distribution of the residues over the triglycerides to be random within the constraints of Table 5.3, the most abundant one would be PPO, making up about 2 mol %. It should be stressed, however, that these calculations are based on assumptions that may be reasonable but are not proved. Moreover, the distribution of residues over the positions, as shown in Table 5.3, is not quite certain and probably varies between lots of milk.

Position of the fatty acid residues in the triglyceride molecules affects physical properties, particularly crystallization, and it determines which fatty acids are predominantly liberated by enzymatic hydrolysis. On the other hand, position has little effect on chemical reactivity. In any case, triglycerides are not very reactive; at room temperature, oxidative attack of the double bonds may occur; at higher temperatures, keto and hydroxy groups may react.

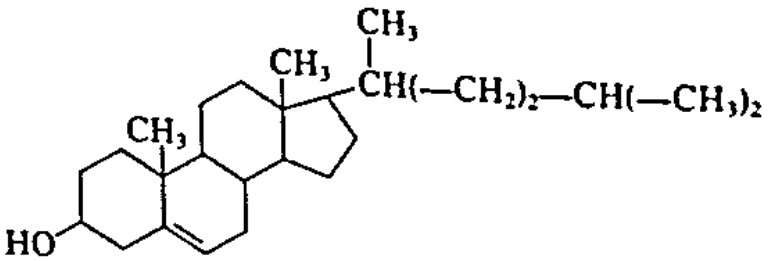
The triglycerides are very apolar and not surface active. They act as a

In all of them, a fatty acid residue is bound to the –NH group; on average, these lipids contain very long-chain fatty acids. (See Table 5.1.) In sphingomyelin, a phosphocholine group (as in lecithin) is bound to the terminal oxygen; it is thus a phospholipid. In the cerebrosides or glycosyl ceramides, a sugar group is bound. This is mostly glucose, and in milk lipids also lactose. The gangliosides are only a minor fraction of milk fat; they have several ketoses, including N-acetylneuraminic acid bound to the terminal oxygen. (See Figure 6.5.)

The properties of the sphingolipids are comparable to those of the phosphoglycerides. All of the compound lipids are found mostly in the same places. The distribution between fat globule membrane and milk plasma (presumably in lipoprotein particles) is for all classes similar, but not exactly equal. Note that there are no compound lipids in the fat globule core.

### 5.2.4. Unsaponifiable Lipids

These consist largely of cholesterol ($C_{27}H_{45}OH$):




A small fraction of the cholesterol is esterified (so this is in fact saponifiable), but authors disagree as to how much. There are also conflicting results on the other, minor, sterols in milk. The sterols are very apolar, but they easily associate with phospholipids.

There are numerous other lipids in milk in very small quantities. These include pigments (mainly carotenoids), vitamins (mainly A, D, and E), antioxidants (mainly tocopherols), and squalene. They are all predominantly dissolved in the core of the fat globules. The appendix gives a list of such compounds. (See Table A.4.) Depending on the feed of the cow, the analytical techniques available, and the diligence of the analyst, the list could be stretched to great length.

## 5.3. THE FAT GLOBULE MEMBRANE

Each fat globule of milk is surrounded by a thin protective layer, usually called a membrane. Its composition and properties are completely different from either milk fat or plasma. It functions to prevent the fat globules from flocculation and coalescence, and it protects the fat against enzyme action.

Properties of the membrane are also of great practical importance, partly for the same reasons. All interactions between fat and plasma must take place through the membrane. Its total area is considerable (some 80 $m^2 \cdot liter^{-1}$ of milk), and it contains reactive substances and enzymes. Consequently, the membrane largely determines such deteriorative reactions as lipolysis and autoxidation.

### 5.3.1. Origin

From the cytological aspects of milk synthesis, it will be clear that the nascent fat droplets inside the cell have a thin layer around them and that they become enrobed in a typical bilayer cell membrane when leaving the cell. (See Sections 2.1 and 2.4.3.) One may perceive these "layers" in electron micrographs of globules just expelled from the cell. The fat globule membrane thus largely derives from the outer membrane (plasmalemma) of the apical part of the cell or from that of the Golgi vesicles. Also, the composition, largely phospholipids and a wide variety of (glyco)proteins, is like that of outer cell membranes.

This does not imply that the fat globule membrane is identical to the apical cell membrane, nor that it resembles it in structure. Electron microscopic studies, though often difficult to interpret, show considerable rearrangement of the membrane shortly after its formation, and for the most part no clear structure is observable. We may envision the following processes:

1. Part of the more apolar substances from the apical cell membrane dissolves into the core. This is probably the case for sterols and carotenoids. This situation is not surprising. In the native state, the cell membrane is bordered at both sides by an aqueous environment. After enrobing the fat droplet, one side comes very close to a strongly apolar material. Now, we should not consider a membrane to be static. Its molecules are continuously in motion, both in tangential and radial directions, and minor ripples go over the surface. Considering the very short distance to the fat core, molecules can reach the latter. Thermodynamic conditions then determine whether they dissolve in the fat or stay in the membrane. Amphiphilic (i.e., surface-active) molecules will stay predominantly. Though the fat globule membrane does not originate as an adsorbed layer of surface-active molecules, as was envisaged earlier, this does not imply that the laws of surface chemistry will not hold.
2. Polar or hydrophilic substances may go to the plasma, either by dissolution or by vesiculation (i.e., formation of small, microsome-like particles of some 10–20 nm diameter that subsequently are dislodged from the fat globule). There is both chemical and electron-microscopical evidence for these processes, but the amounts of substances involved are uncertain. It may well be that the alterations discussed

under number 1 above induce a change in structure of the membrane which, in turn, leads to the loss of more polar substances. But part of the loss may be caused by the cooling that is commonly applied in membrane investigations.

3. Substances from the milk plasma may become adsorbed onto the fat globules. Since there are so many surface-active components available, this assumption appears plausible. There is at least one membrane protein that is probably identical to at least part of one of the proteose peptones (component 3) of milk serum. But we should be careful in the interpretation of proteins and possibly other substances becoming associated with the fat globules. At low temperature, cryoglobulins adhere to the globules (Section 14.3), and this may easily double the amount of protein associated with the fat globules. Leukocytes may swallow fat globules (phagocytosis) and then remain with the cream. Partial denuding of fat globules leads to adsorption of plasma proteins.
4. Chemical changes, mostly caused by enzyme action, may lead to loss of some membrane substances and to adsorption of others. Particularly lipolysis may have such effects, since highly surface-active molecules are formed that may displace other substances from the globule surface. Proteolysis of membrane proteins also takes place. Other changes occur on processing.

Compound lipids and some other substances that are typical of the fat globule membrane are also found in milk plasma. They may derive partly from the membrane. It should be realized, however, that even in a well-skimmed milk, small fat globules are still present, representing some 5% of the total membrane surface, and this should not be mistaken for plasma materials. In freshly drawn, uncooled milk, about 80% of the lipid phosphorus is in the fat globules, while it is usually only 60% in a more "average" milk.

The plasma phospholipids apparently occur in lipoprotein particles, even fairly large ones (probably some 10–50 nm in diameter). They consist of vesicles shredded from the fat globule membrane, and of remnants of microvilli (typical elongated particles) and cytoplasmic organelles, probably from the endoplasmic reticulum. Microbial and white blood cells also contain compound lipids; consequently, plasma lipid content increases with mastitis.

### 5.3.2. Composition

The above discussion points to difficulties in determining membrane composition. An even greater problem is posed by defining the membrane. The outer boundary can be defined by including everything that moves with the fat globules. We can remove plasma substances by "washing" the globules (i.e., diluting cream with water or a buffer and reseparating it). After this is

repeated a few times, "pure" fat globules are left, but part of the membrane substances are lost in the washings However, no definition of the inner boundary can be made operational Numerous authors have isolated preparations from washed globules that they arbitrarily called "fat globule membrane " The isolation procedure usually involves cooling, hence partial crystallization of the fat, and the creation of new interfaces This causes loss of material and contamination with core fat Particularly, the often listed "high melting triglycerides" result from contamination with triglyceride crystals

By contrasting the composition of cream and skim milk, taking into account the specific surface area of the fat globules in both liquids, the composition of the membrane can be determined fairly unequivocally, for components that are absent from isolated fat For other substances, this method is questionable (e g , for cholesterol and carotenoids) or not feasible at all (particularly for neutral triglycerides) The latter also occur in outer cell membranes, though in small quantities Hence, they are probably part of the membrane (i e , present amidst phospholipid molecules) But assigning a numerical value to the abundance of neutral triglycerides in the membrane makes little sense

We should thus interpret the compositional data given in Table 5 4 with

**Table 5.4. Estimated Average Composition of Natural Fat Globule Membranes**

| Components | mg per 100 g Fat Globules | mg per $m^2$ Fat Surface | Percentage of Membrane |
|---|---|---|---|
| Protein | 900 | 4 5 | 48 |
| Phospholipids | 600 | 3 0 | 33 |
| Cerebrosides | 80 | 0 4 | 4 |
| Gangliosides | 20 | 0 1 | 1 |
| Cholesterol | 40 | 0 2 | 2 |
| Neutral glycerides | + | + | ? |
| Hydrocarbons | 20 ? | 0 1 | 1 |
| Ribonucleic acid | + | + | ? |
| Carotenoids + Vitamin A | 0 04? | $2 \times 10^{-4}$ | – |
| Fe | 0 3 | $15 \times 10^{-4}$ | – |
| Mo | 0 05 | $2 \times 10^{-4}$ | – |
| Cu | 0 01 | $0 5 \times 10^{-4}$ | – |
| Water | 200 ? | 1 0 | 11 |
| Total | >1860 | >9 3 | 100 |

caution. We have given more weight to results from creaming experiments than to those on isolated preparations. The latter, however, are indispensable for further study and characterization of components.

Most of the proteins of the membrane are highly specific. Their composition is about as intricate as that of the plasma proteins, but not nearly as well known. (See also Section 6.5.) There are at least 10 different species with molecular weights from 50,000 to 150,000. They are predominantly glycoproteins, among which are sialoglycoproteins. Per 100,000 molecular weight, they contain an average of about 60 mol hexose, 30 mol hexosamine, and 6 mol sialic acid. Some have a very high glucide content. Some are water soluble; some are not. In general, membrane glycoproteins have, besides highly hydrophilic glycosylated regions, strongly hydrophobic regions, which are needed for binding to the lipids of the membrane. Several proteins of the fat globule membrane are metalloproteins, including some enzymes and cytochromes.

The membrane contains at least 10 different enzymes. (See Section 7.2.) This, again is not surprising since cell membranes contain numerous enzymes. Typical and abundant are alkaline phosphatase and xanthine oxidase; each makes up about 10% of the membrane proteins.

The lipids need little further elaboration. The sphingolipids in the membrane contain, on the average, longer-chain fatty acid residues than those in the plasma. The amounts of di- and monoglycerides and fatty acids are uncertain, but because of their surface activity their presence in the membrane is to be expected. The amount of cholesterol reported varies widely.

Numerous minor components may be found. But many of these (e.g., various metals and glucides) are in fact parts of larger molecules, particularly of proteins. The membrane undoubtedly contains some water, primarily bound to its proteins. The quantity given in the table is a rough estimate.

Membrane composition is variable—between lots of milk, between globules in one lot of milk, and between sites on one globule. But it is difficult to evaluate the degree of variability. Differences in amount of a membrane material recovered may merely reflect differences in specific surface area of the globules. Considerable differences in the ratio of components have been found, but even these may not be considered an exact representation of membrane composition, since the individual components may be lost to different extents. Nevertheless, some kind of variability of the membrane must be the cause.

### 5.3.3. Structure

It will be clear from Section 5.3.1 that membrane structure is unknown. There are no well-defined concentric layers, but part of the original cell membrane structure may remain. Such membranes have a distinct "sidedness" (i.e., some lipids and proteins are specific for the inner side and some for the outer side of the cell membrane). There is clear evidence that at least

some of this sidedness is retained in the fat globule membrane. Some phospholipids are largely unreactive to enzymatic hydrolysis when whole globules are treated with phospholipases. Some enzymes are fairly inactive when they are part of the fat globules. But there is also clear evidence, largely by radioactive labeling, that much of the original sidedness of the cell membrane is lost.

The interfacial tension of natural fat globules is very low, 1-2.5 $mN \cdot m^{-1}$. This is lower than can be achieved by adsorption of membrane components at an oil–water interface. It implies that the membrane substances form a highly condensed and somewhat ordered surface layer.

It often is assumed that the membrane contains particles or thickened sections. There is fair evidence that lipoprotein particles are indeed present, though the number seems variable. Anyhow, membrane thickness is markedly uneven. From Table 5.4, an average layer of 8–9 nm is calculated; but thickness varies from place to place. Probably, the membrane is also "hairy"; some molecular hydrophilic chains (probably from glycoproteins) protrude for some distance (maybe about 10 nm) into the plasma.

### 5.3.4. Changes Caused by Processing

Manufacturing processes applied to milk include several treatments that may alter membrane composition and properties.

Cooling (e.g., to 4°C) is a very common treatment, often already applied on the farm. It leads to a migration from fat globules to milk plasma of phospholipids (about 1/5), xanthine oxidase, natural Cu (about 1/3), proteins, and probably other substances. Judging from $H_2S$ production when heating cream and skim milk separated before and after cooling, cooling causes more than half of the membrane proteins that give rise to $H_2S$ to be released from the globules. The cause of these changes is unknown. It may be that the weakening of hydrophobic bonds at low temperatures is involved, but the changes appear to be largely irreversible. It may also be that crystallization of part of the fat is somehow responsible. It is known that fat crystallization is essential in producing the temperature-induced changes in rate of lipolysis and in thickening of cream ("rebodying"), which phenomena must be reflections of changes in the membrane.

Cooling causes adsorption of cryoglobulins onto the fat globules. It is not known whether other substances are adsorbed.

Agitation may have several effects. It may cause coalescence of fat globules, which leads to release of membrane material, since fat surface area decreases; the amount released is probably about proportional to the decrease in surface area. When air is beaten in, fat globules come into contact with air bubbles and lose part of their membrane as it spreads over the air–water interface. (See Section 15.2.)

Fat globules may become damaged, losing part of their membrane (e.g., by contact with air bubbles). They will subsequently acquire a coat of plasma

proteins at the damaged spot. This will always happen to some extent, and undoubtedly it starts during the milking process. When fat globules are disrupted, thereby increasing their surface area, protein adsorption is considerable. It happens, for instance, during evaporation of milk and, naturally, during homogenization. By far the greater part of the surface of homogenized globules is covered with plasma proteins, particularly casein. The fully synthetic fat globules in recombined milk do not even contain remnants of the original membrane.

Damage to the fat globules in nonpasteurized milk will also cause lipolysis, which in turn may further affect the membrane. Phospholipases from bacteria (e.g., *Bacillus cereus*) may alter membrane composition considerably and cause coalescence of fat globules.

Heating is accompanied mostly by agitation or vigorous streaming, and this may cause coalescence of fat globules, particularly at higher temperatures, as well as some disruption. Heating, as such, causes changes in the membrane proteins, probably some denaturation. The release of $H_2S$ has already been mentioned; it takes a few minutes at 80°C or a few seconds at 100°C to produce a noticeable quantity. It is not known for certain whether substances are released from the membrane on heating. There is evidence that substances are adsorbed, probably whey proteins. A considerable fraction of the Cu in milk plasma goes to the fat globules on heating of milk, for instance at 80°C.

Acidification causes some casein to precipitate onto the fat globules. Cu added to the plasma, but not the natural Cu as secreted in the milk, migrates partly to the fat globules on acidification.

## 5.4. THE FAT IN MILK PRODUCTS: MODIFICATION

Since lipid composition differs among the physical fractions of milk (core fat, membrane, plasma) and since milk products differ in the relative abundance of these fractions, their lipid composition varies. Table 5.5 gives some examples. Naturally, lipolysis will alter the composition; its effects depend on whether it occurred before or after separation (by centrifuging or by churning) and on the pH during separation.

The triglycerides in buttermilk (that is to say, true buttermilk obtained by the churning of cream) have a lower melting range than those of whole milk fat, which is reflected, for instance, in a higher degree of unsaturation of the fatty acid residues. The differences can be explained from the processes occurring during churning. (See Section 15.3.) Much of the fat in buttermilk is very finely dispersed. Data on fat globule size in various products are given in Section 14.1.1.

Another milk product is "butter oil". This can be made in various ways. When prepared from fresh butter, by carefully melting and separating the oil layer, its composition closely resembles that of the "core" of the fat

**Table 5.5 Approximate Content of Various Lipids in Some Milk Products**

| Product | Percentage of Product (w/w) | | | |
|---|---|---|---|---|
| | Total Fat | Phospho-lipids | Sterols | Free Fatty Acids |
| Milk | 4. | 0.035 | 0.013 | 0.008 |
| Skim milk | 0.06 | 0.015 | 0.002 | 0.003 |
| Cream | 10. | 0.065 | 0.03 | 0.017 |
| Cream | 20. | 0.12 | 0.06 | 0.032 |
| Cream | 40. | 0.21 | 0.11 | 0.06 |
| Buttermilk[a] | 0.4 | 0.07 | 0.005 | 0.002 |
| Buttermilk[b] | 0.6 | 0.13 | 0.011 | 0.002 |
| Butter | 81. | 0.25 | 0.21 | 0.12 |

[a]From cultured cream of 20% fat.
[b]Same, 40% fat.

globules. If it is to be called "anhydrous milk fat," it should contain at least 99.8% of milk fat and not more than 0.1% water.

The butter oil also may be obtained by washing cream (i.e., diluting with water and reseparating) and then drying it (e.g., in an evaporator or a spray dryer). The fat-insoluble membrane components (protein, part of the compound lipids) are removed by filtering or centrifuging the oil. But part of the compound lipids, particularly phospholipids, now become dissolved in the fat.

Ghee generally is made in a similar way. Cream or butter is heated in a vat until all water has evaporated, and the insoluble parts are removed. The heating produces several components, such as lactones and methyl ketones. These give ghee its typical taste, which varies according to the source and pretreatment (e.g., culturing of the cream).

All of these processes leave the physical properties of the fat essentially unaltered; they are listed in the appendix, Table A.21. Milk fat can be fractioned by partial crystallization and separation, and this yields fractions with different properties. (See also Section 5.7.) Another consequence is that fat-soluble components, like cholesterol, vitamins, and carotenoids (hence color), become concentrated in the liquid fraction.

Milk fat can be modified chemically, but the products can no longer be called milk fat. Hydrogenation saturates double bonds and increases the high melting proportion of a fat, hence its firmness; this is why the process is often called hardening. Interesterification can be achieved by heating the fat in the presence of a catalyst (e.g., sodium methoxide). It is also called

randomizing, since the distribution of the fatty acid residues over the positions of the glyceride molecules becomes random. For milk fat, interesterification results in a higher melting range. *Cis–trans* isomerization has a similar effect on its melting range. None of these chemical modifications is applied presently to milk fat on a commercial scale.

## 5.5. LIPOLYSIS

Lipolysis of milk fat leads to an increase in the concentration of free fatty acids, and this may give the milk product a rancid or soapy taste. The extent of lipolysis usually is expressed as the acidity or acid degree value of the fat in millimoles of free fatty acid per 100 g of fat. Acid degree values greater than 1 usually are considered to be undesirable in milk; at this point, rancidity may become perceptible by taste. Usually, milk does not become rancid spontaneously, though the milk of some cows does. In other words, there is much variability in susceptibility to lipolysis. On the other hand, lipolysis always can be induced in fresh milk by vigorous agitation.

The lipolytic enzyme of milk is a lipoprotein lipase (E.C. 3.1.1.34). Its normal function is to liberate fatty acids from lipoproteins and chylomicrons of the blood, so that these acids can be resorbed by, for instance, the glandular epithelium of the udder. The glycerides to be attacked are associated with proteins and compound lipids, and the enzyme can only act on its substrate when a cofactor is present. Blood always contains cofactor; it is an apoprotein that is part of the high-density and very low-density lipoproteins. It is a relatively small peptide, molecular weight ~ $10^4$. The cofactor is not needed for the enzyme to hydrolyze free glycerides or even some other carboxyl esters; it is needed to bring the enzyme molecule into contact with its substrate when the latter is present as or covered with lipoproteins. The enzyme thus can act at an oil–water interface. The enzyme molecule is surface active and is strongly adsorbed onto such an interface.

Optimal temperature is 37°C, optimal pH ~ 8.5, and divalent cations (e.g., $Ca^{2+}$) stimulate lipase action. The enzyme is strongly inhibited by its products, particularly long-chain fatty acids, probably because it binds them strongly. The initial turnover rate can be very high, greater than 3000 $s^{-1}$ (this means that more than 3000 molecules of fatty acid can be "produced" by one enzyme molecule in 1 s). Most milk contains 1–2 mg of enzyme per liter (i.e., 10–20 n*M*). If it were completely free to act, the mentioned turnover rate would suffice to make milk rancid in 10 s, but this never happens.

In the case of milk and milk products, there are numerous complications in the action of lipase. Some of the most important ones are:

1. Physicochemical conditions in milk, such as pH and ionic composition, are suboptimal. Particularly, the pH is much below the optimum.
2. The enzyme is bound largely to the casein micelles. This considerably diminishes the concentration of free enzyme, hence its ac-

tivity Dissociation of the enzyme from the casein micelles depends on largely unknown factors, and presumably varies Factors 1 and 2 cause the turnover rate in milk to be reduced to some 100 $s^{-1}$, which can induce perceptible rancidity in about 5 min, under conditions that are otherwise optimal (induced lipolysis)

3. The natural fat globule membrane protects the triglycerides effectively against enzyme attack The interfacial tension of the globules is so low (< 2 5 mN $m^{-1}$) that the enzyme cannot penetrate the membrane to gain contact with the fat If the membrane is damaged and locally replaced by plasma proteins, the interfacial tension is increased and the protection impaired This is what happens when lipolysis is induced by homogenization or foaming (See below ) It will happen often to some slight extent during normal handling of milk
4. The apoprotein cofactor alone is not effective in inducing the enzyme to attack natural fat globules But when the lipoproteins that contain the apoprotein are added in sufficient quantities, lipolysis follows The simplest explanation would be that the complex of enzyme with lipoprotein can lower the interfacial tension sufficiently to penetrate the membrane Blood serum added to milk thus causes lipolysis, though there are large differences among cows, with respect to the milk as well as the blood (See Figure 5 1 ) The apoprotein alone, however, is effective as an activator for lipolysis of fat globules that are not covered by natural membranes
5. Milk contains inhibitors (besides the casein micelles), probably in variable quantities Part of the inhibitors are dialyzable and part consist of low molecular weight proteins, among which is the pro-

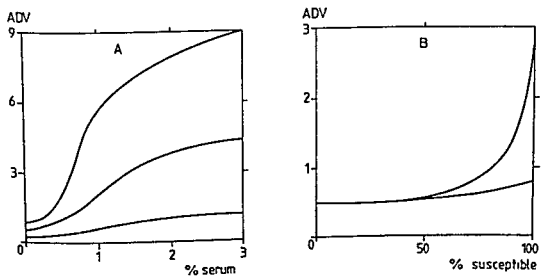



Figure 5 1 Some observations on lipolysis in nonagitated fresh milk kept for 24 h at 4°C ADV = acid degree value as mmol per 100 g fat Different lines pertain to milk from different cows (A) Effect of adding bovine blood serum (B) effect of mixing susceptible with normal milk Mainly after unpublished results of A Jellema

teose–peptone component 3. The proteins probably act only as inhibitors for enzyme attack on natural fat globules. The inhibition can be overcome by sufficient lipoprotein activator. Incidentally, some apoproteins also act as inhibitors.

6. Milk probably contains variable quantities of unknown stimulators (besides the lipoproteins). There is thus a subtle balance between inhibitors and stimulators. This may affect the enzyme as such, the cofactor, the binding of enzyme to substrate and to casein micelles, and the binding of the fatty acids formed. Serum albumin can bind fatty acids, but it is questionable whether such binding significantly reduces product inhibition in milk.
7. Temperature affects the partitioning of potential enzyme activity between fat globules and plasma. Probably, this scarcely concerns the enzyme as such. Some observations point to the cofactor lipoprotein being adsorbed onto the fat globules at low temperature. (Incidentally, cold agglutination of milk fat globules probably also involves adsorption of a lipoprotein onto fat globules. See Section 14.4.)
8. The enzyme is unstable and slowly loses its potential activity in milk. Different authors have obtained somewhat conflicting results, but instability increases with temperature and with decreasing pH. Probably, the enzyme is more stable when it is bound—for instance, to the casein micelles. Oxidation appears to be involved, since adding reducing substances and removing oxygen enhance stability, and addition of, say, 0.02% $H_2O_2$ causes considerable inactivation. Exposure to light rapidly inactivates the enzyme. Proteolytic degradation of the enzyme also may play a part. At room temperature, the potential lipase activity of some lots of milk may be reduced to 80% after 3 h, or to 20% after 24h. But in other lots of milk, the enzyme may be more stable.
9. The enzyme is inactivated by heating, such as 20 s at 75°C. (See Section 7.3.) The cofactor is much more heat stable.
10. Milk may contain other lipolytic enzymes. It was long assumed that fresh and normal milk contained various active lipases, but this has been refuted. Milk contains other esterases, but they are not active against triglycerides in fat globules. Colostrum contains a true lipase (i.e., not a lipoprotein lipase), but normal milk does not. But lipase from bacteria and from leukocytes may be present. It also has been assumed that milk contains a triglyceride synthase since acid degree values, if high, have been observed to decrease on standing.

All of these complications imply that experimental results on lipolysis in milk and milk products should be interpreted with care; conditions may have varied in an unknown way, and various pitfalls may not have been foreseen.

There is hardly any correlation between the potential lipolytic activity of a milk (e g , measured in a milk to which heparin has been added to free the enzyme from the casein micelles and using a tributyrin emulsion as a substrate) and the actual activity (i e , the quantity of free fatty acids formed in the milk on standing )

Susceptibility of the milk to lipolysis varies widely among cows Presumably, this is caused by differences in quantities of activators (cofactor) and inhibitors When a "susceptible" milk is mixed with a normal (i e , non-susceptible) one, the degree of lipolysis of the mixture varies by no means linearly with the mixing ratio (See Figure 5 1 ) This points to an excess of inhibitors in "normal" milk If this were not so, mixed milk would become rancid far more often

The optimal conditions for lipolysis in milk may differ markedly from those in experiments in vitro (enzyme + cofactor + suitable substrate) Optimal temperature is mostly much below 37°C, about 15°C or even lower This may be caused by enzyme inactivation at higher temperatures and by better binding of the cofactor to the fat globules at lower temperatures or by other factors, compare the temperature-induced activation, below The relation between activity and pH also is altered For instance, the ratio between activity at pH 6 6 to that at 8 5 is much higher than in experiments in vitro

It often is assumed that spontaneous lipolysis is caused by a relatively large quantity of activator or cofactor being transferred to the milk from the blood Indeed, addition of bovine blood serum (also of pasteurized blood serum) to milk boosts lipolysis (See Figure 5 1 ) Small additions have little effect It also has been assumed that conditions that favor passing of blood components to the milk would correlate with enhanced lipolysis, and it is indeed found that mastitis and estrus lead to a somewhat increased fat acidity It also increases, often markedly, with stage of lactation, but an even better correlation is found with milk yield When the latter falls below 3 kg per milking, the probability of lipolysis becomes much higher Feeding rations of low quality also may increase lipolysis in the milk

Substrate activation can be done in three ways homogenization, foaming, and temperature manipulation The first two of these methods are very similar, and they always work, though there are quantitative differences among lots of milk The explanation must be that part of the fat globule surface becomes coated by plasma proteins or other substances, and that this surface coat cannot protect the fat as the natural membrane does Figure 5 2A shows that initial lipolysis can be very rapid Consequently, milk should always be pasteurized before it is homogenized, to avoid rancidity It has been assumed that the largely casein-bound lipase becomes partly adsorbed onto the fat surface during homogenization or foaming But this cannot be the explanation, since mixtures of raw milk (or skim milk) and homogenized pasteurized milk show extensive hydrolysis (See Figure 5 2B ) Now, the homogenized fat globules, which act as the substrate, cannot have acquired

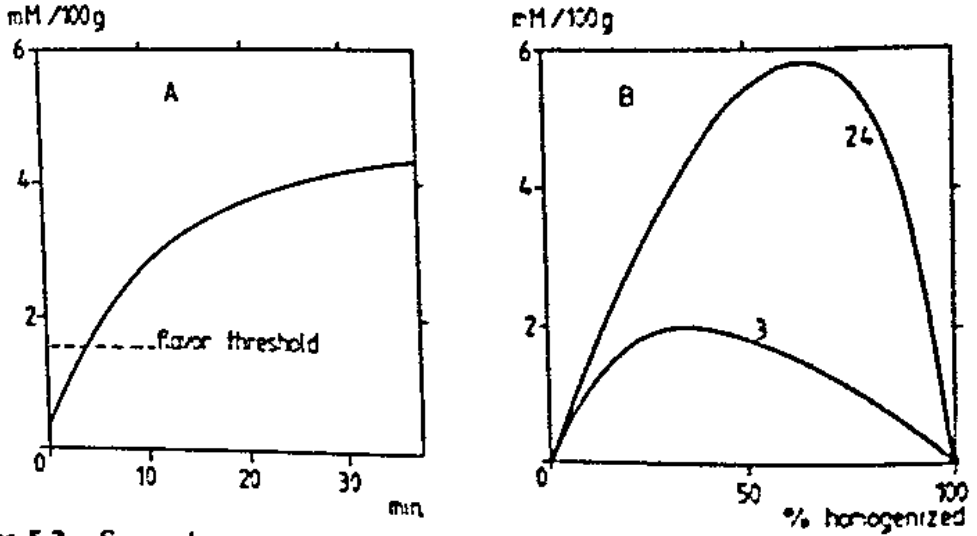



**Figure 5.2.** Some observations on lipolysis (as mmol fatty acids per 100 g fat) induced by homogenization. (A) Development of fat acidity after homogenizing raw milk and keeping it at 37°C; (B) mixtures of raw milk and homogenized pasteurized milk; increase in fat acidity after 3 and 24 h at 15°C. From various sources.

active lipase in their surface coat during homogenization. Figure 5.2 also shows that adding only a few percentages of raw milk to pasteurized homogenized milk, or vice versa, may cause noticeable lipolysis.

Lipolysis often can be induced by temperature manipulation (e.g., by cooling milk to 5°C, warming it to 30°C, and cooling again to 15°C), but the effect is rather variable. The explanation is uncertain. At least part of the effect must somehow be caused by changes in fat crystallization; possibly, the fat globule membrane is damaged. But other factors, such as the binding of the cofactor lipoproteins to the globules, may be involved.

Kinetic aspects of lipolysis in milk have not been studied in detail. The initial rate is about proportional to the fat surface area that is susceptible. But lipolysis rate soon levels off (Figure 5.2A), probably because of product inhibition. Figure 5.2B shows that at low concentration of homogenized milk (i.e., little substrate, much enzyme), the reaction soon slows down. The fatty acids formed will adsorb onto the fat–plasma interface, where they inhibit the enzyme. The partition equilibrium between fatty acids dissolved in the fat and adsorbed onto the interface will be attained very rapidly in any one globule (within 0.1 s). But equilibrium throughout the whole milk may take a long time to establish, since the fatty acids (particularly those with long chains, which are the strongest inhibitors) are so insoluble in water. The explanation may be that with a high concentration of substrate and a low one of enzyme (Figure 5.2B, right-hand side), lipolysis goes on to a higher level of fatty acids, though the initial rate is lower.

Problems caused by lipolysis (e.g., development of a rancid taste) are often the result of a combination of factors. Too intensive agitation (particularly when air is beaten in) of milk of which a high proportion comes from animals that are in late lactation or have mastitis or are poorly fed, may cause an off-flavor before the milk is pasteurized. If considerable growth of

psychotrophic bacteria has taken place, the milk often contains heat-stable bacterial lipases. They may even partly survive UHT treatment (e.g., heating at 140°C for 5 s). When the product is kept for a long time, even very little residual lipase may cause rancidity.

When is milk rancid? Most people taste it when the fat acidity is above 1.4 mmol per 100 g. But this is the acidity as measured, and that implies for most methods that one-fourth to one-third of the free fatty acids are not recovered, since they are in the plasma. Therefore, the true acidity at the threshold of taste is higher. The $C_4$ to $C_{12}$ acids are especially prominent contributors to detectable rancidity. Experimental results on the threshold concentration are not consistent, as it appears to be affected by several factors; it is probably lowest for caprylic acid ($C_{8:0}$; e.g., 10 ppm in water). But the lower their molecular weight, the greater the proportion of the fatty acids in the aqueous phase, and there they can be tasted best.

The pH is an important variable, since it strongly affects partitioning between the phases and since the undissociated acids give the rancid taste. (See Section 5.2.2.) Sour-cream buttermilk, for instance, has a much lower concentration of free fatty acids than sweet-cream buttermilk. The latter may still not taste rancid, but if it is acidified it probably would; since there is little fat present, most fatty acids will stay in the plasma, causing rancidity.

Finally, it should be mentioned that the flavor caused by free fatty acids is not always undesirable. In certain types of cheese (blue cheese, aged cheddar, or Gouda), it is even wanted.

## 5.6. AUTOXIDATION

Unsaturated fatty acids, whether free or esterified, can be oxidized. With some simplification, three groups of reactions can be distinguished.

1. *Initiation.* It generally is assumed that oxygen molecules in the singlet state ($^1O_2$) can oxidize a CH–group directly while shifting the double bond and forming a hydroperoxide:

$$^1O_2 + —CH=CH—CH_2— \rightarrow —CHOOH—CH=CH— \qquad (5.1)$$

   Normal (i.e., triplet) oxygen cannot do this, but it occasionally can be transformed into singlet oxygen by radiation (e.g., light); $^1O_2$ can, moreover, be produced by some other reactions. (See Section 11.5.) Presumably, a suitable catalyst (e.g., a heavy metal ion) can transform the hydroperoxide (denoted as ROOH) into a free radical (e.g., ROO·).

2. *Propagation.* Now, a chain reaction can set in, involving triplet oxygen:

$$\begin{aligned} &\rightarrow ROO\cdot + RH \rightarrow ROOH + R\cdot \\ &\quad R\cdot + {}^3O_2 \rightarrow ROO\cdot \end{aligned} \qquad (5.2)$$

These reactions can keep themselves going, producing hydroperoxides, hence the name autoxidation.

3. *Termination.* Free radicals also can react with each other, giving R–R or R–OO–R, thus terminating the reaction. This gives rise to polymerization. (Highly unsaturated oils like linseed oil thus form a gel on oxidation.)

Antioxidants can interfere with the reactions, usually by reacting with the radicals, thus blocking the chain reaction. However, they are consumed in this process. Many fats contain natural antioxidants, most tocopherols. Numerous synthetic antioxidants have been made and are often employed in food products.

The hydroperoxides, which are the main autoxidation products, have no flavor. But they are fairly unstable and usually break down to form numerous reaction products, mainly $C_6$ to $C_{11}$ unsaturated carbonyl components (i.e., ketones and aldehydes) and some alcohols and acids. (See, for example, Figure 18.4.) Some of the carbonyls, particularly the alk–2,4–dienals (R'–CH=CH–CH=CH–COH) have a very strong flavor (i.e., they have a very low threshold concentration for flavor perception). The resulting off-flavors usually are called "rancid" outside the dairy field (oxidative rancidity).

The more double bonds a fatty acid has, the quicker it autoxidizes. The initial rates of peroxide formation of esters of oleic, linoleic, and linolenic acid are in the proportion of 1:30:80, for instance. Conjugated double bond pairs are more reactive than nonconjugated ones. Consequently, the reaction products depend on fatty acid composition. Since the various reactions moreover, may depend in a different way on such conditions as temperature and redox potential, it is difficult to predict which components will be formed and at what rate.

Figure 5.3 gives a general picture of the common course of events. Note the induction period; it is longer for a higher concentration of antioxidants and a lower temperature.

Milk lipids in various products are subject to autoxidation. In milk and buttermilk, off-flavors may develop within a day. In long-keeping products

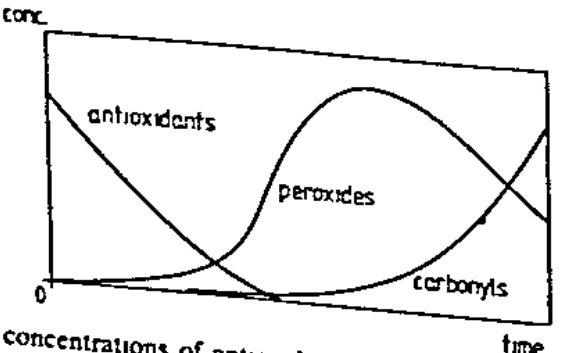



**Figure 5.3.** Relative concentrations of antioxidants, hydroperoxides, and carbonyl components during autoxidation of an unsaturated fat. (Very schematic, not to scale.)

like whole milk powder and butter, the period for off-flavor development is usually a matter of months or even years.

The kind of off-flavor may differ with product and conditions. This is largely caused by differences in flavor components formed by autoxidation, but also by interaction with the other flavoring substances present. In milk, we usually speak of "oxidized" or "tallowy" flavor. Sometimes, a "cardboard" flavor is observed; this is probably caused by oxidation of phospholipids. In sour-cream buttermilk, this may lead to a metallic flavor. For milk powder, the terms "oily," "fatty," or "tallowy" are mostly used. Butter becomes "oily" and, if strongly oxidized, "fishy" (though the flavor actually resembles that of some marine oils like train oil or cod liver oil, not that of fish). The flavor caused by autoxidation is not always undesirable. For example, slight oxidation contributes to the typical aroma of buttermilk. (See also Section 18.3.)

Autoxidation in liquid milk products usually starts with the phospholipids. They have highly unsaturated fatty acid residues (Tables 5.1 and 5.2), and they are in contact with the main catalyst, Cu. After autoxidation is in progress, the triglycerides are also attacked. If Cu content is very high, triglycerides can become oxidized in the absence of phospholipids.

The role of Cu is crucial and depends primarily on its concentration in the fat globule membrane. It is necessary to distinguish between natural Cu (i.e., Cu present in the milk before milking) and Cu entering by contamination, which we will call "added" Cu. Contamination can come from the surface of the udder (dirt always contains Cu), from milking and processing equipment, and from water. Milk is one of the most efficient fluids known for removing ionic Cu from surfaces. Copper is largely bound by the milk proteins.

Of natural Cu (usually 20–25 $\mu g \cdot kg^{-1}$ of milk; in early lactation up to 100 $\mu g$), part is in the membrane, the amount being fairly constant at 10 $\mu g$/100 g fat globules. Added Cu (mostly between 20 and 500 $\mu g$/kg) is much more active as a catalyst than natural Cu. In fresh milk, 1–10% of added Cu goes to the fat globules, the percentage increasing with the quantity added. Cu associated with the globules may increase with processing. (See below.) In aqueous systems, Cu is particularly active as an oxidative catalyst against triglycerides if proteins and phospholipids are present. Other metal catalysts, notably Fe, become inactive in the presence of proteins; consequently, they play no role in autoxidation in milk products (except possibly in butter oil).

Fat oxidation in milk also can be induced by light, particularly light of short wavelengths. Putting a 1-liter bottle of milk for 10 min in direct sunlight usually suffices for a tallowy flavor to develop within 12 h. The light-induced oxidized flavor is to be distinguished from the "sunlight flavor" that can develop in (skim) milk on exposure to light, and in which the lipids play no role. (See Sections 11.5 and 18.3.)

Several milk substances are involved in the autoxidation reactions. Ascorbic acid is essential for Cu-induced oxidation in milk, but large quantities

may inhibit it by lowering the redox potential, which is a factor in autoxidation. Some metalloproteins, notably milk peroxidase and xanthine oxidase, promote autoxidation, but not by their enzymatic action. The cytochrome of the fat globule membrane may be involved in the initiation (perhaps by formation of singlet oxygen). Autoxidation is inhibited by tocopherol, though its concentration in milk is low. It may, however, be predominantly in the fat globule membrane, and its abundance would then be on the order of 20 $mol \cdot mol^{-1}$ of Cu. The concentration of tocopherol in milk varies with the feed of the cow, and this is negatively correlated with proneness to oxidation.

At any rate, there are large variations in susceptibility to develop an oxidized flavor among individual cows' milk. Early lactation milk is particularly prone to oxidized flavor. Apparently, the balance between catalyzing, activating, and inhibiting factors is variable. Some authors hold that some samples of milk may develop an oxidized flavor spontaneously, while others maintain that some added Cu (or exposure to light) is always needed, though the amount of Cu needed varies widely between samples, from 5 to 500 $\mu g \cdot kg^{-1}$. Contamination with as little as 5 $\mu$g Cu per kilogram of milk seems hardly avoidable in practice.

Several *conditions* affect the autoxidation reactions, hence the development of off-flavors:

1. *Water Activity.* It makes a large difference whether the product contains free water (milk, cream, butter) or not (milk powder, anhydrous milk fat). In the latter case, ascorbic acid is not needed to cause autoxidation, the tocopherols naturally present do not act as antioxidants, and phospholipids, if dissolved in the fat, do not promote but rather inhibit oxidation. Water can be an inhibitor; in milk powder, autoxidation rate decreases sharply with increasing water activity.
2. *Temperature.* The rate of autoxidation increases with increase in temperature and $Q_{10} \approx 2$. But in raw or low-pasteurized milk, the situation is reversed; the lower the temperature, the more quickly off-flavor develops. The cause of this peculiarity is not clear. In UHT milk (e.g., heated for 5 s at 140°C), the temperature dependence is "normal."
3. *Oxygen Level.* If the oxygen pressure is below 10 kPa (= 0.1 atm), which means an oxygen concentration in the fat of about 10 $mg \cdot kg^{-1}$, autoxidation rate becomes proportional to oxygen level. Such low concentrations of $O_2$ can only be achieved in fermented milks and cheese, or in hermetically sealed packages. In the latter case, available oxygen may be consumed before an off-flavor develops.
4. *Acidity.* The rate of autoxidation strongly increases with decreasing pH (the optimal pH ≈ 3.8). The main cause is probably the partition of Cu between plasma and fat globules. At pH 4.6, as much as 30–40% of added Cu goes to the globules.

5. *Salt Content.* This is mainly important in butter. In sweet-cream butter, added salt decreases the rate of autoxidation; in cultured-cream butter (e.g., pH 5.0), the rate increases.

*Processing* may affect the development of oxidized flavors considerably, and often it is necessary to optimize process conditions to ensure good keeping quality:

1. Cooling of the milk (e.g., keeping it at 5°C for a few hours) causes nearly half of the natural Cu in the membrane to move to the plasma; the change is apparently irreversible.
2. Heating causes many relevant changes. Some of the added Cu moves to the fat globules. This is noticeable after heating for 15 s at 60°C, and it is considerable after 15 s at 90°C. The Cu content of the membrane may increase by several-fold. The oxidation-promoting properties of the xanthine oxidase and lactoperoxidase proteins are enhanced when they are denatured by heat. Heating for 20 s at 75°C causes roughly 40% of the xanthine oxidase to become denatured (as measured by loss of enzyme activity). These effects may be the cause for the marked increase in proneness to autoxidation by low pasteurization. But more intense pasteurization gives a large decrease in autoxidation rate. This may be caused by the exposure of –SH groups, caused by heat denaturation of $\beta$-lactoglobulin. (See Section 10.3.) Free $H_2S$ also is formed on heating (presumably from proteins of the fat globule membrane), and this may strongly diminish the activity of $Cu^{2+}$, considering the extremely low solubility product of CuS ($\sim 10^{-45}$ mol$^2$·kg$^{-2}$). Possibly, Maillard products also contribute to the marked increase in stability toward autoxidation by heating the milk, say, for 20 s at 95°C. Finally, heating for 15 s at 83°C or higher largely prevents the transport of Cu from plasma to globules on acidification. This is of great practical importance when making butter from cultured cream.
3. Separation can, in principle, be done before or after the milk has been kept cold, though in practice in many countries milk is separated only after cooling. Heating can be applied before or after separation, to the milk or to the cream. These variables affect the distribution of Cu between skim milk and cream, as follows from items 1 and 2 above. Fat content of the cream will affect Cu content of cultured-cream butter markedly; the higher the fat content of the cream, the lower the Cu in butter.
4. Homogenization of milk very much diminishes its proneness to autoxidation, whether induced by Cu or by light. This effect must result from the change in surface coat of the fat globules, but the explanation is unknown. Recombined fat globules need very much Cu to induce autoxidation.

5. Addition of antioxidants may enhance stability to autoxidation considerably, but this usually is not allowed by law. Nor is it usually needed if the milk and its products are properly handled, processed, and stored. But a serious problem arises with whole milk powder; to give it a good keeping quality, the milk should be heated intensely, and this may produce unwanted properties, like a "cooked" flavor.

## 5.7. CRYSTALLIZATION

Melting and crystallization of natural fats are complicated phenomana, and this is certainly true for milk fat with its very wide range of different triglycerides. (See Section 5.2.) Milk fat crystallization is of great practical importance, as it largely determines the physical stability of the fat globules (Section 14.2) and the consistency of high-fat products (Section 16.4).

### 5.7.1. Melting Range

Every individual triglyceride has a sharp melting point. In fact, it can have up to three melting points. (See Section 5.7.4.) But, for the sake of simplicity, we will consider here only its final melting point, which is closely correlated with the melting points of its constituent fatty acids. These increase with chain length and saturation. (See Table 5.2.) But other variables also affect the melting point:

Odd or even number of carbon atoms; odd ones on the average melt ~ 5 K lower.
Branching, a decrease by 1–40 K.
Position of double bond; may make a difference of up to 20 K.
*Trans* double bond; 20–30 K higher than *cis*.
Conjugated *cis* double-bond pair; ~ 25 K higher than nonconjugated.
One hydroxyl group; 5–50 K higher.

Besides the fatty acid composition, the position of residues in a triglyceride molecule determines its melting point. A multicomponent fat thus has a melting range that depends on the distribution of the fatty acids among the triglyceride molecules. For instance, a mixture of tripalmitate and trioleate behaves much differently from a randomized triglyceride mixture with palmitic and oleic acid residues. Interesterification of milk fat also alters its melting range.

The melting points of the triglycerides in milk fat range from roughly −40°C to 72°C. This does not imply that this is the melting range of milk fat; its final melting point is usually ≈ 37°C. The higher-melting triglycerides

dissolve in the liquid fat For a mixture of two triglycerides with widely different melting points, the solubility $x$ (as mole fraction) of the highest melting one at temperature $T$ is given by

$$R \ln x = \Delta_f h \left( \frac{1}{T_f} - \frac{1}{T} \right) \tag{5 3}$$

where $\Delta_f h$ is its molar enthalpy of fusion or melting heat (for many triglycerides between 100 and 200 kJ), and $T_f$ is its melting point (degrees Kelvin) Assuming that tristearate ($\Delta_f h$ = 205 kJ mol$^{-1}$) is the final melting triglyceride in milk fat, its concentration would be predicted as 3 × $10^{-4}$ mole fraction, which is a reasonable result The equation does not hold precisely for the components in milk fat, but serves to explain why any one triglyceride will melt or crystallize over a temperature range The superposition of the ranges for all triglycerides gives the melting range of milk fat

An example is given in Figure 5 4 There are, however, large differences, primarily caused by differences in fat composition, but also by differences in treatment, particularly temperature history, as will become clear below Solid fat content at 0°C may range from about 45% to 90%, or the temperature at which 50% of the fat is solid may be between −5°C and +20°C Final melting point is rarely far from 37°C Solidification curves (i e , solid fat content as a function of temperature when lowering the temperature) may be much different, because of supercooling (See Section 5 7 2 ) Most literature data on solid fat contents give only an apparent content, because of the analytical methods employed (dilatation or thermal analysis), this will become clear below

For most purposes, *crystalline fat* and *solid fat* can be taken as synonyms

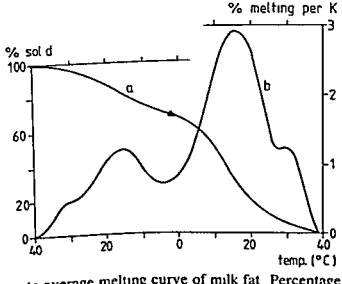



**Figure 5.4** Approximate average melting curve of milk fat Percentage of the fat being solid (a) and percentage of the fat melting per K temperature difference (b) as a function of temperature Fat kept for 24 h at −40°C before melting Mainly after H Mulder and P Walstra *The Milk Fat Globule* (Wageningen Pudoc 1974)

But "solid" also refers to consistency in common parlance. For example, milk fat at 5°C looks like a solid, but it contains maybe 50% liquid fat. Consequently, it is difficult to separate a partially crystalline fat into a liquid and a solid portion. The latter always contains much liquid fat. This severely curtails attempts to fractionate milk fat into high and low melting portions by letting it crystallize at a temperature within its melting range (say at 10°C) and separating the fractions.

An important practical variable—deriving from differences in solid fat content—is the firmness of the fat, particularly in butter. Other factors also affect firmness. These aspects are discussed in Section 16.4.

### 5.7.2. Nucleation

A substance cannot crystallize unless nuclei have formed—that is, tiny embryonic crystals, just large enough to escape dissolving again. A small crystal may dissolve, because the solubility of a small particle increases when its radius of curvature decreases. (See Section 12.1.3.) Homogeneous nucleation (i.e., the formation of nuclei in a pure liquid) requires a considerable supercooling. In milk fat, a supercooling of about 35 K below the final melting point is needed.

In practice, nucleation is usually heterogeneous. Most liquids contain a number of small contaminating particles. At the surfaces of some of these, nucleation can take place, and this happens at a higher temperature ( or less supersaturation) than in homogeneous nucleation. Such particles are called catalytic impurities. Usually, their number (i.e., the number of impurities that can become catalytic for nucleation) increases with supercooling.

In milk fat, if present in bulk (i.e., as a continuous mass), a supercooling of less than 5 K usually suffices to induce nucleation, hence crystallization; this concerns the highest melting triglycerides. As soon as crystals of these have been formed, they act in turn as catalytic impurities for the nucleation of other triglycerides. Since the various triglycerides give very similar crystals, the one can easily nucleate at the surface of the other (this is called epitaxy), and a very small supercooling (e.g., 1–2 K) suffices. Milk fat in bulk thus shows little hysteresis between solidification and melting curves over much of its range, if there was sufficient time for crystallization. (See Figure 5.5A.) But if the fat is finely divided, as in fat globules, the situation may be very different. In bulk fat, one catalytic impurity per milligram would suffice to ensure rapid crystallization. But in milk, 1 mg of fat is divided among some $10^8$ globules, in homogenized milk among at least $10^{11}$ globules. In each of those, at least one nucleus must be formed, and now the number of catalytic impurities may easily become limiting. Consequently, supercooling must be deeper and hysteresis can be considerable. (See Figure 5.5B.) Naturally, the magnitude of this effect depends on globule size: the smaller it is, the deeper the supercooling. If globule volume is $v$ and the

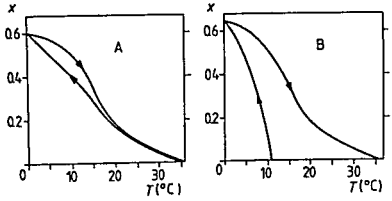



**Figure 5 5.** Proportion of fat being solid ($x$) after 24 h cooling to temperature $T$ (After keeping it at 0°C it was warmed again ) (A) Milk fat in bulk, (B) the same fat in recombined milk, i e , in small globules Example after P Walstra and E C H van Beresteyn, *Neth Milk Dairy J* 29 (1975) 35–65

number of catalytic impurities per unit volume is $B$, then the proportion of the fat in globules containing one or more catalytic impurities $y$ is given by

$$y_{max} = 1 - \exp(-vB) \qquad (5\ 4)$$

And $y_{max}$ is then also the maximum proportion of fat present in globules in which the fat can partially crystallize Naturally, $y_{max}$ depends on temperature, since $B$ does For milk fat, $B$ roughly doubles for each 1 75 K lowering of temperature Figure 5 6 gives examples of $y_{max}$ as a function of temperature and globule size

Nucleation, particularly if heterogeneous, does not occur instantaneously but takes time, depending on nucleating volume (i e , globule volume) and temperature In practice, it is almost complete for milk fat in globules after about 30 min at 5°C, 1 0 h at 10°C, 2 5 h at 15°C, and 6 0 h at 20°C (This does not include the time needed for crystal growth ) Nucleation also depends on fat composition A main variable is lipolysis, as more fat is hydrolyzed, less deep supercooling is needed Presumably, micelles of mon-

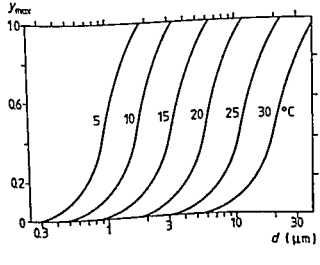



**Figure 5 6** Fraction of the fat present in globules (diameter $d$) that eventually will contain solid fat ($y_{max}$) after keeping at the indicated temperatures Example after P Walstra and E C H van Beresteyn *Neth Milk Dairy J* 29 (1975) 35–65

oglycerides are the main catalytic impurities in globular milk fat, and their number increases with lipolysis.

### 5.7.3. Crystal Growth

In most cases, the crystals initially formed are platelets whose length:width:thickness ≈ 4:2:1. Their concentration would be on the order of $10^9$ per milligram of fat. Their length usually becomes 0.1–2.0 $\mu$m.

As is common with crystallization, rapid and deep cooling of milk fat leads to many nuclei, hence small crystals. Particularly in fat globules, where the fat can be supercooled easily, fat crystals are very small. In bulk fat, they are often larger, and changes in crystallization conditions (see below) may lead to very large ones. Growth of milk fat crystals is very slow. Each large and flexible triglyceride molecule takes a long time before obtaining the right position and conformation to fit into the lattice of a growing crystal. Moreover, there are very many competing molecules, which are so similar to those in the crystal that they almost fit into its lattice. They have to diffuse out again, and this may have to happen numerous times for each vacant site before a properly fitting molecule can occupy it.

For isothermal crystallization of milk fat at 25°C, it generally takes 1–2 h before half of the final amount is crystallized. This $t_{0.5}$ is roughly halved for each 5–6 K temperature decrease. Crystallization rate, however, depends on many conditions, such as crystal surface area (hence size) and the rate of removal of the heat of crystallization.

### 5.7.4. Polymorphism

Triglycerides, like most molecules with long aliphatic chains, can crystallize in three polymorphic modifications, denoted as $\alpha$, $\beta'$, and $\beta$. Each modification is characterized by its crystal lattice type (i.e., the mode of packing of the molecules), not by the geometrical form (habit) of the crystals. An important feature is the distance between the chains, which can be determined by X-ray diffraction, as the so-called short spacings. These are fairly constant, regardless of fat composition.

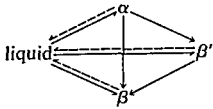
Some examples are in Table 5.6. The melting points increase in the order $\alpha$, $\beta'$, and $\beta$, as do the melting heat and the density of the crystals. This implies that closeness and intricacy of fit of the molecules increase and their freedom of motion decreases. The differences also imply that the melting heat and the volume expansion caused by melting (usually called melting dilatation) vary, and these properties are used commonly to determine solid fat content. The outcome thus depends on which polymorph is predominant. Moreover, it increases with molecular weight and, thus, melting point of the triglyceride. Consequently, these methods give only an apparent (and, because of the dependence on molecular weight, usually too high) solid fat content.

**Table 5.6. Polymorphism of Triacylglycerides**

| Polymorph[a] | $\alpha$ | $\beta'$ | $\beta$ |
|---|---|---|---|
| Crystalline form | hexagonal | rhombohedral | triclinic |
| Main short spacings (Å) | 4.15 | 3.8; 4.2 | 4.6 |
| Properties of tristearate: | | | |
| melting point (°C) | 55 | 64 | 72 |
| enthalpy of fusion ($kJ \cdot kg^{-1}$) | 160 | 180 | 230 |
| melting dilatation ($cm^3 \cdot kg^{-1}$) | 119 | (132) | 167 |

[a]According to Lutton's classification.

The $\alpha$ and $\beta'$ modifications are not stable. Transitions can take place according to the scheme




where solid arrows denote exothermal changes and dotted arrows show endothermal changes. Other transitions cannot occur.

Nucleation of a fat usually occurs in the $\alpha$ modification. Mostly, after a little while, transition to a stabler polymorph occurs. In milk fat that has just started to crystallize at, say, 5°C, we can observe that warming at first causes melting, followed by a second solidification (in a stabler polymorph), and finally melting occurs. In most fats, the $\alpha$ modification has only a short lifetime, while $\beta'$ may persist longer. In milk fat, $\alpha$ crystals can be very persistent (the reason will become clear below), and at low temperatures we mostly find all three modifications simultaneously. The final melting points are in milk fat approximately 22°C, 30°C, and 36°C for $\alpha$, $\beta'$, and $\beta$, respectively.

### 5.7.5. Compound Crystals

A compound or mixed crystal contains two or more different components (molecular species). In some, the components may occur in all proportions; in others the compositional range is restricted. We can further distinguish solid solutions (the molecules are interchangeable at any location in the crystal) and layer crystals (each layer of one or two molecules thick contains only one type of molecule).

In fats, both types probably can exist. Compound crystals are formed easily in the $\alpha$ modification; since the molecular packing is not very dense, there is some freedom of fitting different molecules into one crystal lattice.

By the same reasoning, compound crystals are less abundant in the $\beta'$ modification. In $\beta$ crystals, the molecules must be very similar, and only small fractions of one kind of molecule can be in the crystal of another. Compound fat crystals are not stable; they tend to slowly rearrange into pure crystals. They have a lower enthalpy of fusion and a lower density than pure crystals.

Milk fat exhibits extensive mixed crystallization, the more so when it is cooled more rapidly to lower temperatures. This probably is caused by the very great number of different, though similar, triglycerides. The final melting temperature of a compound crystal containing many similar triglycerides may be higher than that of each of the molecules separately, though $T_f$ and $\Delta_f h$ of equation 5.3 will be lower. But the total concentration at saturation $x$ is much higher than that of each separate triglyceride. This also may be the cause of the persistence of the $\alpha$ modification in milk fat, since such multicomponent compound crystals can exist only in this polymorph.

Mixtures of triglycerides show a wide range of phase diagrams. The simplest type, two components giving compound crystals over the full compositional range, is illustrated in Figure 5.7. Above the liquidus line there is only liquid, below the solidus there is only solid fat, and in between there are both. For instance, cooling a mixture with composition $a_3$ rapidly from $T_1$ to $T_2$ gives crystals with composition $a_5$, and a liquid phase $a_2$ remains. The mass ratio of solid to liquid is $(a_3-a_2)/(a_5-a_3)$. When the temperature is changed, the composition of both liquid and solid phase will change, but it may take a long time before equilibrium is reached; this is because rearrangement has to take place in the crystalline phase, which is a slow process, the more so as temperature is lower. Further cooling, for instance to $T_3$, will leave the solid phase $a_5$ largely as it is, while the liquid phase $a_2$ will segregate into $a_1$ (liquid) and $a_4$ (solid). Afterward, $a_5$ crystals will change slowly into $a_4$, but the amount of $a_1$ decreases in this process; hence, the proportion of solid fat increases.

In milk fat, the situation is more intricate than that of the binary mixture of Figure 5.7. There are not two but many components; some give compound crystals and others do not; polymorphism is involved. Milk fat certainly does not make one type of compound crystal. But it gives a series of many compound crystals, existing side by side over most of its melting range. In

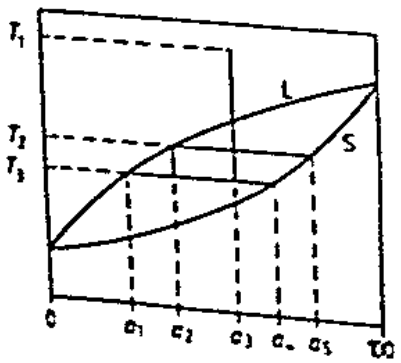



Figure 5.7. Phase diagram of a binary mixture (0–100% $a$), giving the phases as a function of temperature ($T$). L is the liquidus, S the solidus line.

analogy to the simple phase diagram, some qualitative conclusions can be drawn

1. Compound crystals narrow the melting range
2. The temperature at which most of the fat melts depends on the temperature at which solidification took place Cooling in steps may give two melting maxima (See Figure 5 8B )
3. Cooling in steps, or very slow cooling, which is about the same, gives less solid fat than rapid cooling to the lowest crystallization temperature (See Figure 5 8A )
4. Cooling to a lower temperature before bringing to the final temperature gives more solid fat than direct cooling to the latter This is shown in the hysteresis at low temperatures in Figure 5 5A
5. When equilibrium has not been reached (i e , when the fat has not been directly cooled to and kept at one temperature), rearrangement of crystal composition occurs (See Figure 5 8B ) Below 5°C, this may be a matter of months, above 10°C, it may take days or hours After lowering the temperature of partially crystallized milk fat, the amount of solid fat may increase during several days After increasing the temperature (to below the final melting point), it may take up to a half-hour before the decrease in amount of solid fat is finished

The most important consequences of compound crystallization are the extensive slow changes it may evoke Segregation of compound crystals mostly goes along with transition to a stabler polymorph Moreover, large crystals tend to grow at the expense of smaller ones (This is Ostwald ripening, see Section 12 1 3 ) All of these changes are enhanced by temperature fluctuation They cause change in size (usually an increase) and in arrange-

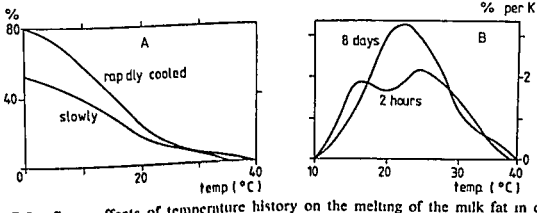



**Figure 5 8** Some effects of temperature history on the melting of the milk fat in cream (Highly schematic ) (A) Percentage of solid fat after rapidly and slowly cooling to −40°C, (B) differential melting rate (percentage of the fat per K temperature difference) in cream cooled in steps i e held a day at 19°C, then 2 h or 8 days at 10°C Mainly after H Mulder and P Walstra *The Milk Fat Globule* (Wageningen Pudoc, 1974)

ment of the crystals. This, in turn, strongly affects rheological properties, as will be discussed in Section 16.4.

## *Suggested Literature*

Most textbooks in biochemistry and in food chemistry give useful details about lipids. A short but good overview of chemistry and physics of lipids is given by

D. Chapman, *Introduction to Lipids* (London: McGraw-Hill, 1969).

Biochemical aspects are in

M. I. Gurr and A. T. James, *Lipid Biochemistry: An Introduction*, 3rd ed. (London: Chapman and Hall, 1980).

Analytical methods are in

L. V. Cocks and C. van Rede, *Laboratory Handbook for Oil and Fat Analysis* (London: Academic Press, 1966).

Several topics in this chapter, particularly the fat globule membrane and crystallization, are treated in more detail in

H. Mulder and P. Walstra, *The Milk Fat Globule: Emulsion Science as Applied to Milk Products and Comparable Foods* (Wageningen: Pudoc, 1974).

Composition and properties of milk lipids are given by

W. R. Morrison, "Milk Lipids," in F. D. Gunstone, ed., *Topics in Lipid Chemistry*, Vol. 1 (London: Logos, 1970), pp. 51–106.

F. E. Kurtz, "The Lipids of Milk: Composition and Properties," in B. H. Webb, A. H. Johnson, and J. A. Alford, eds., *Fundamentals of Dairy Chemistry*, 2nd ed. (Westport: AVI, 1974), pp. 125–219.

P. Walstra and T. J. Geurts, "Milk Fat and Butter," in I. A. Wolff, ed., *Handbook of Processing and Utilization*, Vol. 1 (Boca Raton: CRC Press, 1982).

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S. Patton and T. W. Keenan, *Biochim. Biophys. Acta* 415 (1975): 273–309.

Many aspects of lipolysis are in

W. K. Downey and J. M. Cogan, eds., "Proceedings of the Lipolysis Symposium, International Dairy Federation, Document 86 (1975).

J. H. Moore, ed., "Flavour Impairment of Milk and Milk Products due to Lipolysis," International Dairy Federation, Document *118* (1980).

A review of some variables, particularly processing, on lipolysis is

H. C. Deeth and C. H. Fitz-Gerald, *Austr. J. Dairy Technol.* 31 (1976): 53–64.

A good, though somewhat outdated review of the chemistry of lipid autoxidation is

R. T. Holman, "Autoxidation of Fats and Related Substances," *Progr. Chem. Fats and Other Lipids* 2 (1954): 51–98.

An authorative discussion with special emphasis on kinetics is

T. P. Labuza, *CRC Crit. Rev. Food Technol.* 2 (1971): 355–405.

A shorter discussion is found in several textbooks on food chemistry, such as

L Dugan, "Lipids," in O Fennema, ed , *Food Chemistry* (New York Marcel Dekker, 1976), pp 139–204

Newer views on initiation of autoxidation are reviewed by

M Korycka Dahl and T Richardson, *J Dairy Sci* **63** (1980) 1181–98

Autoxidation of milk lipids is reviewed by

O W Parks, ' The Lipids of Milk Deterioration, Part II Autoxidation," in B H Webb, A H Johnson, and J A Alford, eds , *Fundamentals of Dairy Chemistry*, 2nd ed (Westport AVI, 1974), pp 240–72

The chemical pathways of lipid breakdown during autoxidation, especially of milk fat, are treated by

H T Badings, *Neth Milk Dairy J* **24** (1970) 147–256

General aspects of fat crystallization are in an extensive, though somewhat outdated discussion by

A E Bailey, *Melting and Solidification of Fats* (New York Interscience, 1950)

A more up-to-date but much more restricted review is by

J Hannewijk, A J Haighton, and P W Hendrikse, in H A Boekenoogen, ed , *Analysis and Characterization of Oils Fats and Fat Products* Vol I (London Interscience, 1964), pp 119–30

Polymorphism of fat crystallization is reviewed by

D Chapman, *Chem Rev* **62** (1962) 433 Phase diagrams of triglyceride mixtures are given by J B Rossel, *Adv Lipid Res* **5** (1967) 353

Some fundamental aspects of nucleation and growth of fat crystals are discussed by

M van den Tempel, *S C I Monogr* **32** (1968) 22

Crystallization of milk fat in the emulsified state is treated by

P Walstra and E C H van Beresteyn *Neth Milk Dairy J* **29** (1975) 35–65

# 6

# Proteins

This chapter presents a brief summary of the chemistry and structure of proteins in general and describes the kinds, proportions, and properties of the nonenzyme proteins of bovine milk. Heat denaturation of milk proteins is dealt with in Sections 10.2 and 10.3, enzymes of milk in Chapter 7, casein micelles in Chapter 13, milk protein preparations in Section 17.7, and nutritive value of milk proteins in Section 19.5.

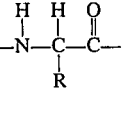
## 6.1. CHEMISTRY AND CONFORMATION OF PROTEINS

The trinucleotide codons of the genetic code specify 19 primary $\alpha$-amino acids and one cyclic secondary amino acid as monomer building blocks of proteins. These are all of the L-configuration, except for glycine, which has no asymmetric carbon. They are listed with abbreviations and some properties in Table 6.1.

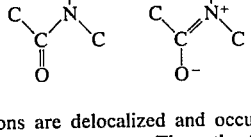
Although the biosynthetic mechanism accommodates only 20 kinds of residues, a number of posttranslational modifications of the side chains occur in vivo in various proteins. In milk proteins the important ones are those mentioned in Section 2.3.2 (i.e., phosphorylation of Ser and Thr; glycosylation of Asn, Ser, and Thr; and oxidation of Cys to CysS-SCys). Furthermore, in many proteins some "tailoring" occurs in vivo by specific cleavage of peptide bonds. Other modifications of side chains of residues can occur in vitro, and some of them are especially important in milk processing (e.g., interaction of $\epsilon$-$NH_2$ of Lys with sugar carbonyls).

The *primary structure* of proteins consists of polypeptide chains of amino acid residues joined "head-to-tail" by peptide linkages. (See Figure 6.1.) Peptide linkages are substituted amides that may be considered to have resulted from interaction of the carboxyl group of one amino acid with the

amino group of the next. The term *amino acid residue* refers to the following structure:



The structure and dimensions of the peptide linkage are depicted in Figure 6.2. The electron distribution over the O, C, and N of the linkage is intermediate between that of two structures:

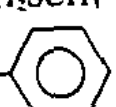


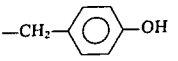
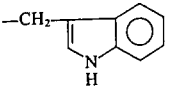
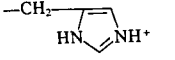
Actually, some electrons are delocalized and occupy a $\pi$-like molecular orbital extending over all three atoms. Thus, the C–O bond is less than double, and the C–N bond has partial double-bond character. Stabilized in this way, the amide link is planar; rotation about the C–N axis would require considerable expenditure of energy. As drawn in Figure 6.2, the $\alpha$ carbons (i.e., the carbons bearing the side chains) are at diagonally opposite corners of the amide plane in the *trans* configuration. This is the predominant form occurring in proteins. The opposite configuration, the *cis* form, is less sterically favorable in polypeptides and occurs only rarely.

Proteins differ from each other in length of polypeptide chains and in the proportions and sequence of residues in them. Generally, the term *protein* is applied to natural polypeptides containing 50 or more residues (insulin contains 51 residues). The sequence of residues in proteins is determined genetically. Sequence differences in a given protein among biological species and within species result from mutations in the DNA codons that have occurred in the course of evolution.

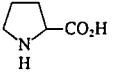
Proteins of various species are said to be homologous if they are considered to have originated from a common ancestor. The most definitive criterion of homology is congruence of amino acid sequence. Homologous proteins usually, but not always, exhibit a common biological function, but coincidence of function also can arise by convergence from different ancestral lines. Closely related homologous proteins have common antigenic determinants and thus exhibit immunological cross-reaction (A reacts with anti-B), but lack of cross-reactivity does not necessarily imply nonhomology. Even within a species small variations in the amino acid sequence of a protein can occur, and these are called *genetic polymorphs*. (See also Section 2.3.2.) Homologs and genetic polymorphs often are detected by electrophoretic

**Table 6.1. Properties of Amino Acid Residues**

| Name of Acid | Symbol | Side Chain | Reactive Group | p$K^a$ | Charge at pH 6.6 | $H\phi^b$ |
|---|---|---|---|---|---|---|
| Glycine | Gly | —H | | | | 0 |
| Alanine | Ala | —$CH_3$ | | | | 3.1 |
| Valine | Val | —$CH(CH_3)CH_3$ | | | | 7.1 |
| Leucine | Leu | —$CH_2CH(CH_3)CH_3$ | | | | 10.0 |
| Isoleucine | Ile | —$CH(CH_3)CH_2CH_3$ | | | | 12.3 |
| Serine | Ser | —$CH_2OH$ | Hydroxyl | | | (−1.2) |
| Threonine | Thr | —$CHOHCH_3$ | Hydroxyl | | | 1.9 |
| Aspartic acid | Asp | —$CH_2CO_2^-$ | Carboxyl | 4.0 | −1 | ~0 |
| Asparagine | Asn | —$CH_2CONH_2$ | Amide | | | |
| Glutamic acid | Glu | —$CH_2CH_2CO_2^-$ | Carboxyl | 4.5 | −1 | ~0 |
| Glutamine | Gln | —$CH_2CH_2CONH_2$ | Amide | | | |
| Lysine | Lys | —$(CH_2)_4NH_3^+$ | $\epsilon$-Amino | 10.5 | +1 | 6.3 |
| Arginine | Arg | —$(CH_2)_3NHC(NH_2)_2^+$ | Guanidine | >12.5 | +1 | 3.1 |
| Cysteine | Cys | —$CH_2SH$ | Thiol | 9.5 | 0 | ~4.0 |
| Methionine | Met | —$CH_2CH_2SCH_3$ | Thio-ether | | | ~5.4 |
| Phenylalanine | Phe | —$CH_2$— | Phenyl | | | 11.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Tyrosine | Tyr |  | Phenol | 9 6 | 0 | 11 9 |
| Tryptophan | Trp |  | Indole | | | 12 5 |
| Histidine | His |  | Imidazole | 6 4 | $+\frac{1}{2}$ | ~2 0 |
| Proline | Pro | (Note *c*) | | | | 10 9 |
| Phosphoserine | SerP | $-CH_2O-PO_3^{2-}$ | Phosphoric acid | 1 5, 6 5 | $-1\frac{1}{2}$ | — |
| Terminal | — | $-CO_2$ | α-Carboxyl | 3 6 | −1 | — |
| groups | — | $-NH_3^+$ | α-Amino | 7 6 | +1 | — |

[a]Approximate intrinsic ionization constant in unfolded peptide chains
[b]Hydrophobicity in kJ/residue according to Tanford

[c]Secondary amino acid 

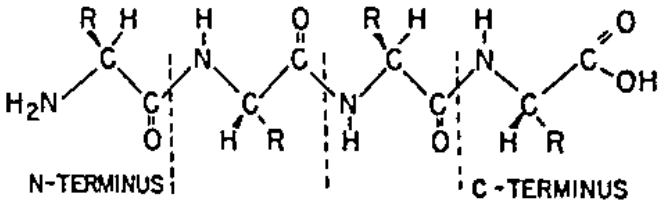



**Figure 6.1.** Structure of peptide chain.

differences, but, of course, polymorphs can arise by substitutions that do not alter net charge.

The three-dimensional structure of proteins is designated *conformation*. Present evidence indicates that conformation of a given protein is specified uniquely by the amino acid sequence. The newly synthesized polypeptide chain assumes the conformation most thermodynamically stable for the environment in which it finds itself, and, in many cases, that conformation is partially fixed by the formation of disulfide bridges. It is convenient to classify the architecture of protein into four structural levels. *Primary structure*, already described, is merely the polypeptide chain of amino acid residues joined by peptide linkages and cross-linked by disulfide bridges, if any; it is the covalent structure. *Secondary structure* refers to the steric relationships of residues close to each other in the linear sequence, particularly where these steric relationships are regular and periodic. The $\alpha$-helix and the $\beta$-pleated sheet are examples of this level of structure. Further folding and coiling gives rise to steric relationships among residues far apart in the linear sequence. These are called *tertiary* (or *supersecondary*) *structure*. The dividing line between secondary and tertiary structure is arbitrary. Proteins are designated *globular* if they are compactly coiled and folded into more or less spherical or ellipsoidal units and *fibrous* if they consist of long stretches of intermolecularly linked polypeptide chains. Many proteins fold into a

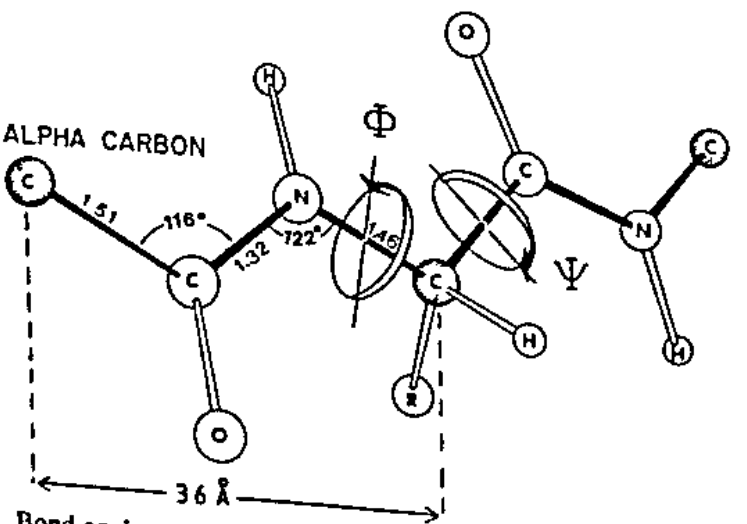



**Figure 6.2.** Bond angles and lengths (Å) and rotational freedom in the peptide bond.

number of compact globular units connected by relatively flexible regions of polypeptide chain; such globular units are called *domains*. Some proteins contain two or more polypeptide chain subunits; the manner in which they are associated is referred to as *quaternary structure*.

The peptide bond is planar; therefore, the polypeptide chain has only two degrees of freedom per residue—rotation around the C$\alpha$–N bond axis, $\phi$, and that about the C$\alpha$–C axis, $\psi$, as shown in Figure 6.2. The arrangement of the polypeptide in space is defined completely by the succession of $\phi$, $\psi$ angles. Many $\phi$,$\psi$ angle combinations are not sterically possible; they bring the side chain groups too close together. The region from $\phi = 20°$–$140°$ is particularly favorable sterically. If the twists at every $\alpha$ carbon are the same, the chain becomes arranged into a helix. Such a helix of repeating subunits can be described by the number of units per turn, $n$, and the distance parallel to the axis traversed per turn, $d$. The product, $nd$, is the pitch, $p$. Both $n$ and $d$ are determined once $\phi$ and $\psi$ are specified. Two important structures in proteins are the right-handed $\alpha$-helix and the antiparallel $\beta$-pleated sheet. Both are regular arrangements stabilized by H-bonds.

The right-handed $\alpha$-helix has $\phi = 132°$, $\psi = 123°$, $n = 3.61$ residues per turn, $d = 1.50$ Å, and radius = 2.3 Å. It is stabilized by H-bonding between the –C=O of residue $i$ and the –NH of residue $i + 4$. This is a particularly stable rodlike structure. Many proteins have extensive helical domains. For example, myoglobin is a protein of 153 residues of which 121 are located in eight $\alpha$-helices ranging in length from 7 to 26 residues. The propensity for forming $\alpha$-helices is influenced somewhat by the side chain groups of the residues, and many attempts have been made to derive general rules relating helix formation to the sequence of residues. These rules are based either on statistical data on the frequency of residues in helical sequences or on their physicochemical properties. Agreement on the propensity for $\alpha$-helix formation of various residues is not complete, but certainly the proline residue, having a cyclic structure and no H to donate to an H-bond, is a "helix breaker." Glycine is rather indifferent, and such residues as Ala, Glu, Phe, His, Ile, Leu, Met, Gln, Val, and Trp have strong tendency to form helices. Helicity is measured experimentally by circular dichroism (the difference in optical activity of the compound to light circularly polarized in opposite directions) and by optical rotatory dispersion (the dependence of the optical rotation of the compound measured with plane-polarized light on wavelength). The most definitive, though very expensive, method to establish helicity is by X-ray diffraction of protein crystals.

The antiparallel $\beta$-pleated sheet has $\phi = 40°$, $\psi = 315°$, $n = 2.00$ residues per turn, $d = 3.50$ Å and radius = 1.0 Å. It consists of polypeptide chains aligned parallel to each other with alternate chains having opposite orientation (i.e., N → C vs. C → N). The chains are hydrogen bonded to each other through –NH···O=C– bonds. The need to make good hydrogen bonds keeps the chains from being fully extended, and thus the structure falls at $\phi = 40°$, $\psi = 315°$, instead of at 0°, 0°. This produces a pleated effect and

pushes the successive side chains out from the sheet on opposite sides. The classic example of a protein having this structure is silk. In this protein long stretches of the chain have the six-residue unit Gly-Ser-Gly-Ala-Gly-Ala repeated many times. Thus, all of the Gly residues will be on one side of the sheet, and all of the Ala and Ser on the other, and the sheets can pack closely together.

A third structure found in proteins is a reverse turn in which the chain bends back upon itself and a hydrogen bond is formed between the –C=O of a particular residue $i$ and the –NH of $i + 3$. They are located mostly at the surface of proteins and primarily involve hydrophilic residues. They are of several different stereoconfigurations having different values of $\phi$ and $\psi$. These turns involve only the four residues $i$, $i + 1$, $i + 2$, and $i + 3$, rather than an extended and repeated H-bonded structure as in an $\alpha$-helix.

Other secondary structures are found in some proteins; the distinctive triple helix of collagen is especially interesting. However, the $\alpha$-helix, $\beta$-pleated sheet, and reverse turn structures, along with random coil regions, seem to be the only ones found in milk proteins, although admittedly the structures of these proteins have not been defined thoroughly.

Folding and coiling of the basic secondary structure of a protein into tertiary (supersecondary) structures is determined mostly by noncovalent interactions of side chains. The main forces involved are hydrogen bonds, ionic bonds, and hydrophobic interactions. As already described, H-bonds between amide N and carbonyl are of major importance in the $\alpha$-helical and $\beta$-pleated sheet structures. Several of the side chain groups also are able to form hydrogen bonds; –OH and =NH groups can act as hydrogen donors, =O, =N–, –S–, and –O– as acceptors. Ionic bonds or salt bridges result from mutual attraction of ionized groups of opposite charge. The Asp and Glu residues and terminal carboxyls are negatively charged, and the Lys and Arg and terminal amino groups are positively charged at physiological pH. The p$K$s of these groups are influenced by environment, and thus salt linkages may be affected by neighboring groups in the structure, particularly hydrophobic ones. Ionization of the imidazole ring of His is especially sensitive to variation with pH in the physiological range, since the p$K$ is about 6. Ionized groups of like charge, of course, will repel each other.

Hydrophobic interactions represent the tendency of nonpolar groups to associate in water. The main cause is that water molecules around a hydrocarbon group are more ordered (i.e., have less entropy) than elsewhere in the solution. When two hydrocarbon groups associate, some of the oriented water molecules are released and become less ordered (increase in entropy); the association of nonpolar groups is driven by this increase in entropy of the water. Moreover, Van der Waals attraction forces tend to be larger between two nonpolar groups than between a nonpolar group and water. Hydrophobic interaction is a major force in ordering the folding of the structure of proteins. Proteins tend strongly to have the nonpolar groups inside the structure and the polar groups on the outside. A large portion (60–80%)

of the surface area that is exposed in the unfolded protein is buried when it is folded.

It is generally felt that in aqueous solvents H-bonds contribute little to the stability of the three-dimensional structure of proteins unless they are inside a tightly folded (i.e., hydrophobic) region. If the protein groups that could make such bonds are in an aqueous environment, they bond with the dipolar water molecules. A similar reasoning holds for salt bridges if the ionic strength of the solvent is low. This further emphasizes the primary importance of hydrophobic interactions in protein structure; the conformation that they establish favors electrostatic and hydrogen bonding.

An important property of a protein is the pH at which the net charge is zero. This point, called *isoelectric point* or *isoelectric pH* and symbolized p*I*, may be determined by interpolation of plots of electrophoretic mobility versus pH or by isoelectric "focusing" in a pH gradient. Such p*I* values depend on the ionic strength; the latter must be specified or the p*I* at zero ionic strength obtained by extrapolation to compare proteins meaningfully. The isoionic point is defined as the pH of no charge in the absence of any ions except $H^+$ and $OH^-$. It may be obtained experimentally by taking the pH of a protein solution deionized by passage through a monobed resin column (a mixture of $R^+OH^-$ and $H^+R^-$ resins). Isoionic point may be calculated from the p*K*s of ionizable groups in the protein (extrapolated to zero ionic strength). Calculated isoionic points are subjected to some error because of uncertainty regarding effects of neighboring groups on the p*K*s.

*Denaturation* of proteins denotes unfolding and uncoiling of the tertiary and secondary structure without rupture of the covalent peptide bonds of the primary structure. Denaturation results from various physical and chemical treatments that disrupt the noncovalent bonds that maintain the secondary and tertiary structure. Among such treatments are heat treatment, high and low pH, organic solvents such as ethanol, and high concentrations of such solutes as urea or guanidine. (See also Table 6.2.) Obviously, heat is the major denaturing agent of milk proteins in milk processing. Denaturation exposes side chain groups that were buried in the native structure, and the process thus causes an increased reactivity of such groups. Increased reactivity of thiol groups of Cys residues is especially important because they can undergo oxidation to disulfide (–S–S–) or sometimes cysteic acid ($-SO_3H$) groups. Furthermore, disulfide interchange can occur in which $RSH + R'S-SR'' \rightarrow RS\text{-}SR' + R''SH$ thus shifting the position of the –S–S– linkages and in some cases forming intermolecular bonds. Denaturation is essentially a reversible process unless oxidation of thiols and disulfide interchange occur. It has also been assumed that partial *trans* → *cis* isomerization of peptide bonds involving the N-terminal side of proline residues may hinder return to the native conformation. In order completely to unfold and uncoil the polypeptide chains of Cys-containing proteins, the disulfides must be split during the denaturation process. A common way of preparing an unfolded protein for investigative purposes is to reduce disulfides and

**Table 6.2. Denaturation of Globular Proteins**

| Agent | Particulars | Probable Cause |
|---|---|---|
| 1. Urea; guanidinium chloride | At high concentration | Breaks H-bonds, weakens hydrophobic interactions. |
| 2. Sodium dodecyl sulfate | At low concentration | Hydrophobic binding to the protein. |
| 3. Mercaptoethanol | pH ≥ 8 | Reduces –S–S– bridges. |
| 4. Ethanol | At high concentration | Dehydrates; weakens hydrophobic interactions; enhances salt bridges. |
| 5. Some salts (e.g., LiBr, $CaCl_2$) | At high concentration | Roughly same as 4 above; specific interactions. |
| 6. High pH | | Electrostatic repulsion; activation of thiol groups. |
| 7. High temperature | | Increased effect of conformational entropy; weakens hydrophobic interactions; secondary reactions. |

alkylate the resulting thiols or to oxidize them to $-SO_3H$ in the presence of denaturing agents. Important physical manifestations of denaturation are increase in specific optical rotation and often decreased solubility and aggregation. The latter may involve the formation of intermolecular disulfide linkages.

## 6.2. PROTEIN COMPOSITION OF MILK

Bovine milk contains about 5.3 g of nitrogen per kilogram. Of this, about 95% is in the form of proteins—approximately 32 $g \cdot kg^{-1}$. About 80% of the milk proteins (i.e., 26 $g \cdot kg^{-1}$) consists of caseins, a group of phosphate-containing milk-specific proteins that precipitate upon acidification to pH 4.6. Almost all casein in milk is present in casein micelles. (See Chapter 13.) Four kinds of polypeptide chains, designated $\alpha_{s1}$-, $\alpha_{s2}$-, $\beta$-, and $\kappa$-caseins, together with some derivatives formed by proteolysis of these chains, are included in the category of caseins. The proteins (6 $g \cdot kg^{-1}$) remaining in solution at pH 4.6 are called *whey proteins (or milk serum proteins)*; they consist of a rather diverse group including $\alpha$-lactalbumin, $\beta$-lactoglobulin, bovine (blood) serum albumin, immunoglobulins, and small molecular weight peptides derived by proteolysis of some of the caseins. All of the proteins named above have been isolated and at least partially characterized. In

addition, milk serum contains a number of so-called minor proteins and a number of enzymes. Other proteins and enzymes are located in the membrane of the fat globules; they amount to about 0.35 g per kilogram of milk.

The principal milk proteins all exhibit genetic polymorphism. All of the variants known at present differ in ionizable residues and were originally detected by electrophoresis of milk proteins from individual cows. In most cases the positions of the substitutions have been revealed by sequencing techniques. Conceivably, other variants not differing in charge remain undetected. Amino acid sequences of the most common genetic variant of each of the six principal milk proteins are presented in the appendix; Table A.7, amino acid substitutions in the other variants are given in Table A.8. Nongenetic variants also occur in some of the milk proteins because of differences in number and location of phosphate and glycosyl groups attached to the polypeptide chains after translation.

Many data are available on variability of total protein content and the relative proportions of casein and whey protein. This variability is discussed in Chapter 9. On the other hand, quantitative data on the concentrations of the individual proteins are scarce. Table 6.3 presents a general view of concentrations, and Table 6.4 lists some properties of the principal proteins.

**Table 6.3. Concentration of Proteins in Milk**

| | Concentration in Milk | | Percentage of Total Protein (*w/w*) |
|---|---|---|---|
| | $g \cdot kg^{-1}$ | $mmol \cdot m^{-3}$ | |
| Total Protein | 33.0 | ~1490 | 100.0 |
| Total Casein | 26.0 | 1170 | 79.5 |
| Whey Proteins | 6.3 | ~320 | 19.3 |
| Fat Globule Membrane Proteins | 0.4 | | 1.2 |
| $\alpha_{s1}$-casein | 10.0 | 440 | 30.6 |
| $\alpha_{s2}$-casein | 2.6 | 110 | 8.0 |
| $\beta$-casein | 9.3 | 400 | 28.4 |
| $\gamma$-casein | 0.8 | 40 | 2.4 |
| $\kappa$-casein | 3.3 | 180 | 10.1 |
| $\alpha$-lactalbumin | 1.2 | 90 | 3.7 |
| $\beta$-lactoglobulin | 3.2 | 180 | 9.8 |
| Blood Serum Albumin | 0.4 | 6 | 1.2 |
| Immunoglobulins | 0.7 | ~4 | 2.1 |
| Miscellaneous (including Proteose–Peptone) | 0.8 | ~40 | 2.4 |

*Note:* Approximate averages from various sources.

## Table 6.4. Properties of Some Milk Proteins

| Property | Caseins | | | | Whey Proteins | | |
|---|---|---|---|---|---|---|---|
| | $\alpha_{s1}$-B | $\alpha_{s2}$-A | $\beta$-A$^2$ | $\kappa$-B | $\alpha$-La-B | $\beta$-Lg-B | Serum Albumin |
| Molecular Weight | 23,614 | 25,230 | 23,983 | 19,023[a] | 14,176[a] | 18,363 | 66,267 |
| Residues/Molecule | | | | | | | |
| Amino acids | 199 | 207 | 209 | 169 | 123 | 162 | 582 |
| Proline | 17 | 10 | 35 | 20 | 2 | 8 | 34 |
| Cys | 0 | 2 | 0 | 2 | 8 | 5 | 35 |
| –S–S– | 0 | ? | 0 | ? | 4 | 2 | 17 |
| Phospho- | 8 | 11 | 5 | 1 | 0 | 0 | 0 |
| Carbohydrate | 0 | 0 | 0 | + | [b] | [c] | 0 |
| Hydrophobicity (kJ/res) | 4.9 | 4.7 | 5.6 | 5.1 | 4.7 | 5.1 | 4.3 |
| Charge | | | | | | | |
| mol % residues | 34 | 36 | 23 | 21 | 28 | 30 | 34 |
| Net charge/residue | −0.10 | −0.07 | −0.06 | −0.02[d] | −0.02 | −0.04 | −0.02 |
| Distribution | uneven | uneven | very uneven | very-uneven | even | even | |
| $A_{280}$ | 10.1 | 14.0[e] | 4.5 | 10.5 | 20.9 | 9.5 | 6.6 |

[a]Exclusive of carbohydrate residues.
[b]A small fraction of the molecules.
[c]O, except for a rare variant (Dr).
[d]Average.
[e]$A_{290}$.

## 6.3. CASEINS

It is difficult to define caseins in a way that includes all proteins belonging to the class and excludes all others. Their common property of low solubility at pH 4.6 serves (at least for bovine milk) as the basis for a rather convenient operational definition. At this pH all of the caseins except some of the proteolytic derivatives precipitate. Their solubility is so much less than that of any of the whey proteins at these conditions that a clearcut separation is possible.

Resolution of the mixture of caseins into individual molecular components for either analytical or preparative purposes is a difficult task, best accomplished by ion exchange chromatography on DEAE-cellulose or hydroxyapatite columns. In such chromatographic separations interactions among casein molecules must be suppressed. This is accomplished by making the medium 6 *M* in urea and either including an agent like dithiothreitol to reduce disulfide bonds or reducing and alkylating the disulfides before chromatography.

Compositionally, the hallmark of the caseins is ester-bound phosphate. All of the casein polypeptide chains have at least one such group per molecule; none of the whey proteins has any. The $\alpha_{s1}$- and $\beta$-caseins contain no cysteine residues; $\alpha_{s2}$- and $\kappa$-caseins each have two. Proline contents of caseins are rather high (17, 5, 17, and 12 mol % in $\alpha_{s1}$-, $\alpha_{s2}$-, $\beta$-, and $\kappa$-, respectively). The distribution of charged groups and proline and cysteine residues is given in Figure 6.3.

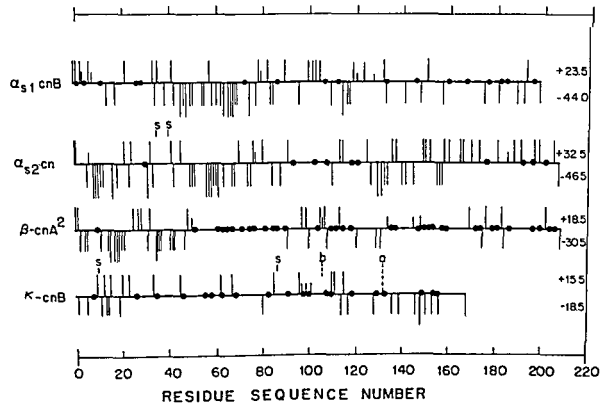



**Figure 6.3.** Location of charged residues (pH 6–7), Pro (●) and Cys (S), in caseins. (a) Location of glucide residue (b) Point of cleavage by chymosin

None of the four kinds of casein has a highly organized secondary structure. None of the various methods of analysis suggests anything more than short lengths of $\alpha$-helix or $\beta$-sheet structure in them. Their ionizable groups are accessible to titration and other side chains to reaction, and neither the reactivity of such groups nor the optical rotation is materially increased in the presence of denaturing agents or by heating. Thus, their conformation appears to be much like that of denatured globular proteins. The large number and rather uniform distribution of Pro residues may help to prevent a close-packed orderly secondary conformation.

A small part of the $\alpha_{s2}$-casein is in the form of a disulfide-linked dimer, and $\kappa$-casein occurs naturally as a mixture of disulfide-linked oligomers. The four caseins differ greatly from each other in charge distribution and in tendency to aggregate in the absence and presence of $Ca^{2+}$ ions.

### 6.3.1. $\alpha_{s1}$-Caseins

The polypeptide chain of $\alpha_{s1}$-casein (Figure 6.3) consists of two predominantly hydrophobic regions (residues 1–44, and 90–199) and a highly charged polar zone (45–89). All but one of the phosphate groups are in the 45–89 segment, and the prolines are distributed at intervals in the hydrophobic segments. Thus, this protein can be visualized as a rather loose flexible polypeptide chain. Self-association of $\alpha_{s1}$-casein depends markedly on its concentration and on the pH, ionic strength, and kind of ion in the medium, but it is relatively independent of temperature. Physical measurements such as light scattering, sedimentation, and viscosity indicate that $\alpha_{s1}$-casein is completely dissociated to a flexible random chain monomer of MW = 24,000 at pH 12, in 3 *M* guanidinium chloride and at neutral pH and 0.01 ionic strength. It associates at neutral pH and higher ionic strength, the extent of association depending on protein concentration; the *n*-mers are in rapid equilibrium. (See Figure 6.4.) It binds about 8 moles $Ca^{2+}$ per mole near pH 7, probably to the ester phosphate groups. It aggregates and precipitates at very low concentrations of $Ca^{2+}$ (7 m*M* $Ca^{2+}$, 28 m*M* NaCl). A small amount of peptides, sometimes called $\lambda$-casein, is present in milk; these appear to originate from proteolysis of $\alpha_{s1}$-casein.

### 6.3.2. $\alpha_{s2}$-Caseins

$\alpha_{s2}$-casein has a remarkable dipolar structure with a concentration of negative charges near the N-terminus and positive charges near the C-terminus. (See Figure 6.3.) Its properties have not been investigated as thoroughly as those of the other caseins, but certainly it binds Ca strongly and is even more sensitive to precipitation by $Ca^{2+}$ than is $\alpha_{s1}$-casein. It self-associates at neutral pH in the absence by $Ca^{2+}$, and the association depends markedly on ionic strength and is at a maximum at an ionic strength of about 0.2. The standard free energy of association is about $-38$ kJ·mol$^{-1}$ at 20°C. The

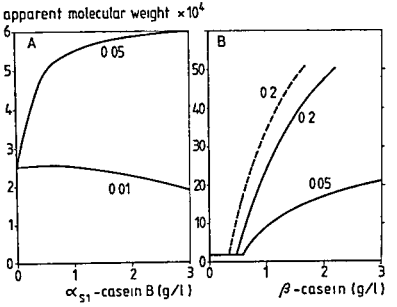



**Figure 6.4.** Association of $\alpha_{s1}$- and $\beta$-casein as a function of concentration. pH 6.6 in $\alpha_{s1}$-, 7.0 in $\beta$-casein. Temperature 21°C, for broken line 24°C. Ionic strength (m*M*) designated on curves. After D. G. Schmidt and T. A. J. Payens, *Surface and Colloid Science* 9 (1976):165.

association, like that of $\alpha_{s1}$-casein, is in rapid equilibrium and leads to polymers of steadily increasing size as the concentration is increased.

### 6.3.3. $\beta$-Caseins

$\beta$-casein has a strongly negatively charged N-terminal portion. (See Figure 6.3.) The net charge of the 21-residue N-terminal sequence is $\sim -12$ at pH 6.6, and the rest of the chain has virtually no net charge. The large number of Pro residues effectively precludes extended helix formation. Thus, the $\beta$-casein molecule is somewhat like that of an anionic detergent with a negatively charged head and an uncharged essentially hydrophobic tail. The outstanding characteristics of the association of $\beta$-casein in both the absence and the presence of $Ca^{2+}$ is its strong dependence on temperature. In the absence of $Ca^{2+}$, only monomer is present at 4°C, but large polymers (20–24 monomers) are formed at room temperature. The association is like that of detergent micelles in that intermediate polymers are not present and in the occurrence of a "critical micelle concentration." (See Figure 6.4.) Removal of the 20-residue C-terminal sequence destroys the ability of $\beta$-casein to associate, suggesting that specific hydrophobic interactions may be involved. $\beta$-casein tightly binds about 5 $Ca^{2+}$ per mole, consistent with its ester phosphate content.

### 6.3.4. $\gamma$-Caseins

A group of caseins designated as $\gamma$-caseins has been known for some time to correspond to C-terminal portions of the $\beta$-casein sequence. These are formed by cleavage of $\beta$-casein at positions 28/29, 105/106, and 107/108 by

the enzyme plasmin. The fragments 29–209, 106–209, and 108–209 constitute the γ-caseins. The smaller fragments resulting from the cleavage appear in the whey when casein is precipitated by acid and constitute part of what has long been designated the proteose–peptone fraction of the whey. Thus, fragments 1–105 and 1–107 were called whey component 5, fragment 1–28 was whey component 8-fast, and fragments 29–105 and 29–107 were whey component 8-slow. Recommended nomenclature for the fragments of β-caseins occurring in milk is given in Table 6.5.

### 6.3.5. κ-Caseins

About one-third of the κ-casein molecules are carbohydrate-free and contain only one phosphate group (SerP-149). At least six other components differing in charge also occur. They have varying numbers of N-acetyl neuraminic acid (NANA) residues and one, at least, appears to have a second phosphate (SerP-127). Three different glycosyl oligomers linked to Thr-133 have been identified. One has the structure shown in Figure 6.5, and the others lack one or the other of the two NANA residues. The N-terminal residue of κ-casein is glutamic acid. In the isolated protein it is present as the cyclic derivative pyroglutamic acid. It is not settled whether κ-casein occurs in this fashion in the milk as secreted or is an artifact produced during the processes of isolation. κ-casein as isolated from milk consists of a mixture of polymers probably held together by intermolecular disulfide bonds; these polymers range in molecular weight from about 60,000 (trimers) to more than 150,000.

κ-casein is rapidly hydrolyzed at the Phe(105)–Met(106) bond by the enzyme chymosin (EC 3.4.23.4), and by other proteases, yielding an N-terminal fragment called para-κ-casein, which contains the two cysteine residues, and

**Table 6.5. Nomenclature of β-Casein Fragments**

| Fragment | Recommended Nomenclature | Former Nomenclature |
|---|---|---|
| 29–209 | β-casein X[a] (f29–209) | $\gamma_1$-casein |
| 106–209 | β-casein X (f106–209) | $\gamma_2$-casein |
| 108–209 | β-casein X (f108–209) | $\gamma_3$-casein |
| 1–105 | β-casein X (f1–105) | Component 5[b] |
| 1–107 | β-casein X (f1–107) | |
| 29–105 | β-casein X (f29–105) | Component 8-slow[b] |
| 29–107 | β-casein X (f29–107) | |
| 1–28 | β-casein X (f1–28) | Component 8-fast[b] |

[a]X designates any of the genetic variants; the specific one should be indicated if known.
[b]Components of the proteose–peptone fraction.

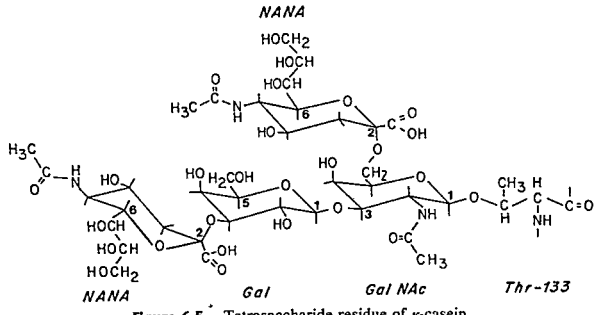



**Figure 6.5.** Tetrasaccharide residue of κ-casein.

a C-terminal fragment of 64 residues called the macropeptide, containing all of the carbohydrate and phosphate groups as well as the genetic substitutions. This reaction, which is the basis of the curdling of milk by rennet and thus of cheese making, is further discussed in Section 13.3.

κ-casein binds about 2 moles $Ca^{2+}$ per mole of protein at neutral pH but differs markedly from the other caseins in its solubility over a wide range of $Ca^{2+}$ concentration. In the absence of Ca and the presence of dithiothreitol (to prevent the formation of disulfide bonds) carbohydrate-free κ-casein polymerizes at pH 7.0 to a spherical particle of 23 nm diameter and MW = 570,000 (30 monomers). These parameters are not influenced greatly by variations in ionic strength above 0.1, but the critical micelle concentration decreases as the ionic strength is raised. The free energy of association per mole monomer is about $-36\ kJ \cdot mol^{-1}$.

The naturally occurring mixture of bovine κ-casein variants polymerizes, as previously mentioned, through –S–S– linkages to subunits containing three to eight monomers. These further polymerize by noncovalent association to particles of about 650,000 daltons. This polymerization is insensitive to concentration of $Ca^{2+}$ and to temperature.

### 6.3.6. Interactions of Caseins

As previously mentioned, all of the caseins tend to self-associate in solution. Many studies have also revealed coassociation of $\alpha_{s1}$-, β-, and κ-caseins in various binary and ternary mixtures. Little such work has included $\alpha_{s2}$-casein. At neutral pH, 37°C, and the absence of $Ca^{2+}$, $\alpha_{s1}$-β-casein copolymers are more stable than polymers of either alone, and $\alpha_{s1}$-κ-casein complexes are more stable than β-κ-casein complexes or κ-casein polymers. The complexes formed are evidently in equilibrium with the monomers. Such complexes, with mean sedimentation constants of about 7.5 Svedberg units

and a considerable range of sizes, are formed in solutions of whole casein and in ternary mixtures of $\alpha_{s1}$-, $\beta$-, and $\kappa$-caseins in the absence of $Ca^{2+}$.

Association of caseins in the presence of $Ca^{2+}$ is considerably more interesting because of its application to the milk system than is association in systems devoid of $Ca^{2+}$. Differences in Ca binding capacities of the caseins have been pointed out previously. Figure 6.6 compares the Ca-binding of $\alpha_{s1}$- $\beta$-, and $\kappa$-caseins. $\kappa$-casein has a remarkable ability to stabilize $\alpha_{s1}$- and $\beta$-caseins against precipitation by $Ca^{2+}$. Typical results are shown in Figure 6.7; $\kappa$-casein can stabilize about 10 times its own weight of $\alpha_{s1}$- or $\beta$-casein against precipitation by Ca. It loses this stabilizing power when cleaved by chymosin. Native $\beta$-casein does not protect $\alpha_{s1}$-casein against Ca precipitation, but, interestingly enough, dephosphorylated $\beta$-casein does so, although not as effectively as $\kappa$-casein. The protective power of dephosphorylated $\beta$-casein is further enhanced by glycosylating it.

## 6.4. WHEY PROTEINS

When milk is brought to pH 4.6, the caseins precipitate. The supernatant contains several proteins that are collectively called whey proteins. Most are globular proteins, subject to heat denaturation. They become less soluble if milk is heated. Some of the smaller proteolytic products from the caseins are included operationally in the whey proteins; they make up the major part of the proteose–peptones. These are not subject to heat denaturation and are partly in the casein micelles at milk pH. Consequently, the whey proteins are not identical to the proteins of rennet whey. Another difference is that the latter include the caseinomacropeptide, resulting from chymosin action on $\kappa$-casein.

Separation of individual components of the whey protein mixture originally was accomplished by fractionations based on differences in solubility and by crystallization. At present the preferred methods of fractionation for

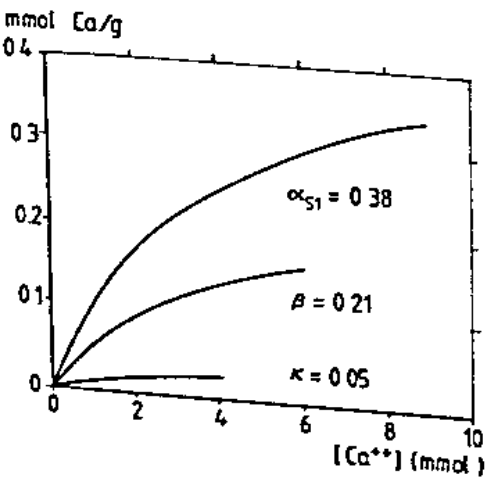



**Figure 6.6.** Binding of Ca by individual caseins at pH 7.4 as a function of $Ca^{2+}$ concentration. Ester phosphate content (mmol · $g^{-1}$ casein) is entered on the curves. After I. R. Dickson and D. J. Perkins, *Biochem. J.* 127 (1971):235.

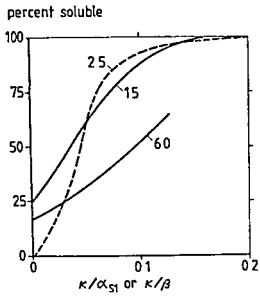



**Figure 6.7.** Effect of κ-casein on solubility (%) of $\alpha_{s1}$-casein (broken line, pH 7.1, 20 m*M* $CaCl_2$, 37°C) and β-casein (pH 7.0, 100 m*M* $CaCl_2$, 30°C). The concentrations of $\alpha_{s1}$- and β-casein (mg · $ml^{-1}$) are designated on the curves. After various sources.

both analytical and preparative purposes make use of gel filtration (which fractionates on the basis of size) and ion exchange (which separates on the basis of charge). Gel filtration is particularly useful for the fractionation of the proteins of bovine whey because they vary so greatly in molecular size. The fact that β-lactoglobulin (MW = 18,300) forms a noncovalent dimer makes it readily separable from α-lactalbumin (MW = 14,200), which does not polymerize.

### 6.4.1. α-Lactalbumin

Two genetic variants, A and B, of this protein exist. They differ by a single substitution, A having Gln and B having Arg at position 10. The B variant is the only one in the milk of European breeds and yaks; both A and B occur in Indian cattle. Some minor forms of bovine α-lactalbumin are revealed by electrophoresis. Some of these contain covalently bound carbohydrate groups; the major components of bovine α-lactalbumin are devoid of carbohydrate. Others of the minor components seem to have fewer amide groups than the major ones, and one minor α-lactalbumin containing three instead of four disulfides has been reported. In total, the minor components do not account for more than 10% of the α-lactalbumin.

The amino acid sequence of α-lactalbumin is similar to that of lysozymes. Indeed, bovine α-lactalbumin B and chicken eggwhite lysozyme have identical amino acid residues at 49 positions, and the four disulfide groups are located identically; they are at 6–120, 28–111, 61–77, and 73–91 in α-lactalbumin. Distribution of charges, Pro and Cys residues, are shown in Figure 6.8. It is considered that α-lactalbumin arose in evolution by gene duplication of an ancestral gene coding for lysozyme. Although the conformation of α-lactalbumin has not been defined completely by X-ray crystallography, preliminary indications are that its structure is similar to that of the well-defined lysozyme. The two proteins must have very different active centers, how-

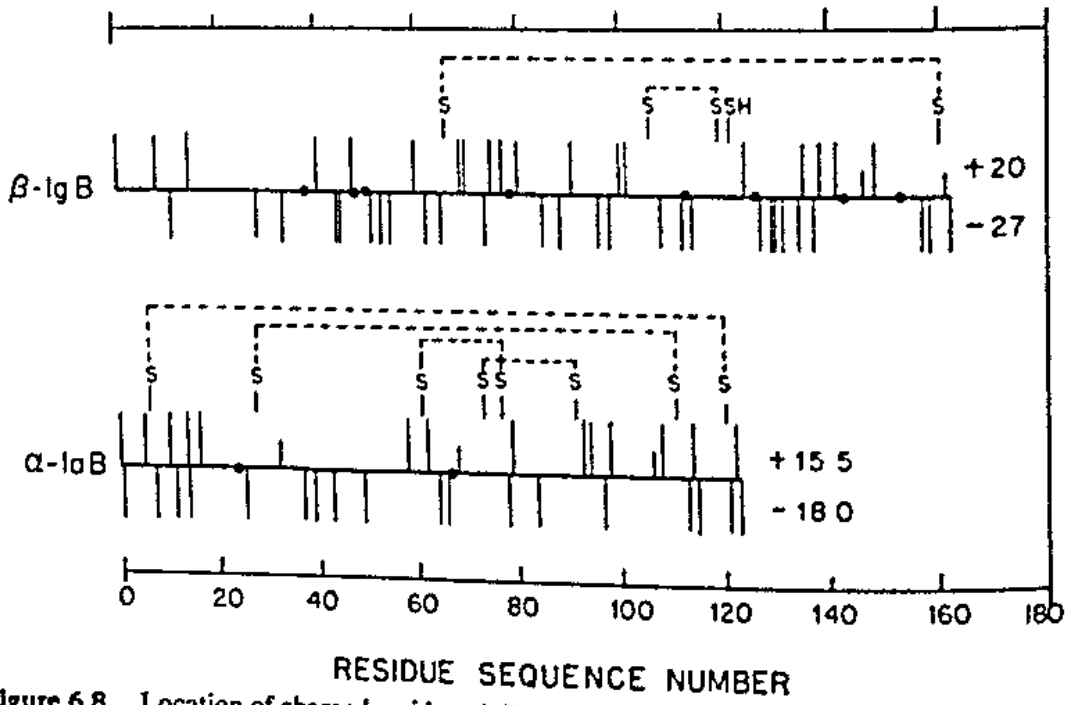



**Figure 6.8.** Location of charged residues (pH 6-7), Pro (•) and Cys (S), in β-lactoglobulin and α-lactalbumin.

ever, since neither has the biological activity of the other; nor do they interfere with each other's activity. The biological activity of α-lactalbumin is interaction with galactosyl transferase to promote the transfer of galactose from UDP galactose to glucose to form lactose. The Michaelis constant $K_m$ (see Section 7.1) for glucose in this reaction is lowered from about 1400 m*M* to 4 m*M* by the presence of α-lactalbumin. The latter should be considered a modifier of the enzyme; it does not appear to participate in the catalytic reaction directly.

It has been shown recently that α-lactalbumin binds two atoms of Ca very tenaciously. In fact it is probable that all preparations of this protein have carried this Ca undetected. Removal of the bound Ca with ethylenediamine tetraacetate renders α-lactalbumin more susceptible to denaturation by heat or guanidine.

### 6.4.2. β-Lactoglobulin

Bovine β-lactoglobulins contain five cysteine residues per mole, of which four are involved in disulfide linkages. Charge distributions and location of Pro and Cys residues are shown in Figure 6.8. One disulfide links residue 66 to 160, and the other links 106 to either 119 or 121. The single free thiol appears to be equally distributed between Cys 119 and Cys 121. The existence of this thiol group is of great importance for changes occurring in milk during heating, as it is involved in reactions with other proteins, notably κ-casein and α-lactalbumin. (See Section 10.3.)

It is likely that considerable portions of the sequence of β-lactoglobulin exist in the α-helix and β-sheet structures. Regions that are most likely helical are residues 21–37, 51–63, 127–143, and 154–159. β-sheet structures are likely in 2–19, 39–43, 76–88, 91–99, and 101–107. Different methods give ambiguous predictions for the sequence 114–125.

β-lactoglobulin exists naturally as a dimer of two monomeric subunits noncovalently linked. When more than one genetic variant is present, hybrid dimers are formed. Dissociation to the monomer occurs below pH 3.4. β-lactoglobulin A associates to form an octamer at pH 4.5 and low temperature.[1] The B variant (which is the predominant one in Western cattle) octamerizes to a much smaller extent.

β-lactoglobulin undergoes a very interesting conformational change as the pH is raised above 6.0. The protein at pH 6.0 is said to be in the N state. Raising the pH causes a transition to another state, designated R, with a molar increase in optical rotation of about 10. This change is associated with release of one proton per subunit from a buried carboxyl and also with changes in the environment of a Tyr and the free Cys group. X-ray analyses of crystals of β-lactoglobulin in the N and R states should define the structural differences between them. To date such analyses have been made only at 6 Å resolution. They show certain features suggestive of α-helices and β-sheets, but the resolution needs to be improved to delineate the entire course of the polypeptide chain and the specific differences between the states.

### 6.4.3. Bovine Serum Albumin

This protein, a major component of blood serum, is synthesized in the liver and gains entrance to milk through the secretory cells, but it comprises only about 1.2% of the total milk protein. Milks of all mammalian species that have been examined (about 200) have a protein component that is electrophoretically indistinguishable from the blood serum albumin of the species. The protein as isolated from bovine milk could not be differentiated from that isolated from bovine blood by methods available in 1950. Since that time little work has been done on the serum albumin isolated from milk; it has been assumed to be identical to that in blood. The protein isolated from blood consists of a single polypeptide chain of 582 amino acid residues. Its tertiary structure reveals three about equal-sized globular domains. It has one free thiol (Cys 34) and 17 disulfide linkages, which neatly hold the protein in a multiloop structure. Several of the bridges are of the double Cys variety in which two Cys residues adjacent in the sequence form disulfide bridges with two other groups. Adjacent Cys residues cannot link to each other by disulfide bonds because of the *trans* structure of the peptide bond. For example, one double Cys bridge involves disulfides between Cys residues 123 and 167 and between 166 and 175. Such double bridges have the effect of pulling three segments of the polypeptide chain close to one another. (There is considerable homology among various segments of the chain in

this protein, and it has been suggested that it arose by repeated duplication of an ancestral gene in the same lineage from which globins were derived.) Serum albumin appears to function as a carrier of small molecules (e.g., fatty acids) that bind to it. Any specific role it may play in the function of the mammary gland is unknown. Furthermore, little is known of its behavior in milk and milk products and its possible influence on their properties.

### 6.4.4. Immunoglobulins

Immunoglobulins are antibodies synthesized in response to stimulation by macromolecular antigens foreign to the animal. They are polymers of two kinds of polypeptide chains, light (L) of MW 22,400, and heavy (H). The latter are of several types, including $\gamma$ (MW 52,000), $\alpha$ (MW 52,000–56,000), and $\mu$ (MW 69,000). Each of the L and H chains consists of a relatively constant and highly variable sequence and appears to be coded for by two genes. The nomenclature of the classes of chains and of antibodies was developed for human antibodies and was extended to other species, including cattle, on the basis of homology of the heavy chains as revealed by immunological cross-reaction with those of humans. IgG1 and IgG2 are each polymers of two light chains and two heavy chains of the $\gamma$ type ($\gamma$1 and $\gamma$2). The chains are joined by disulfide linkages to form two antibody sites, each consisting of the variable portion of an H and L chain. IgG1 and IgG2 have about 2.9% bound carbohydrate and MW of about 150,000. They differ slightly in electrophoretic mobility. (See Figure 6.9.)

IgA and IgM immunoglobulins likewise have the basic structure of two H and two L chains joined by disulfide bridges. In IgA the H chains are of the $\alpha$ type, and in IgM they are of the $\mu$ type. IgA is secreted as a dimer of two of the basic four-chain units joined by a polypeptide of MW about 25,000, called J-component, and associated with another called secretory component, SC. This complex is called secretory IgA (SIgA) and has a MW of about 385,000. The secretory component is a protein of MW about 75,000,

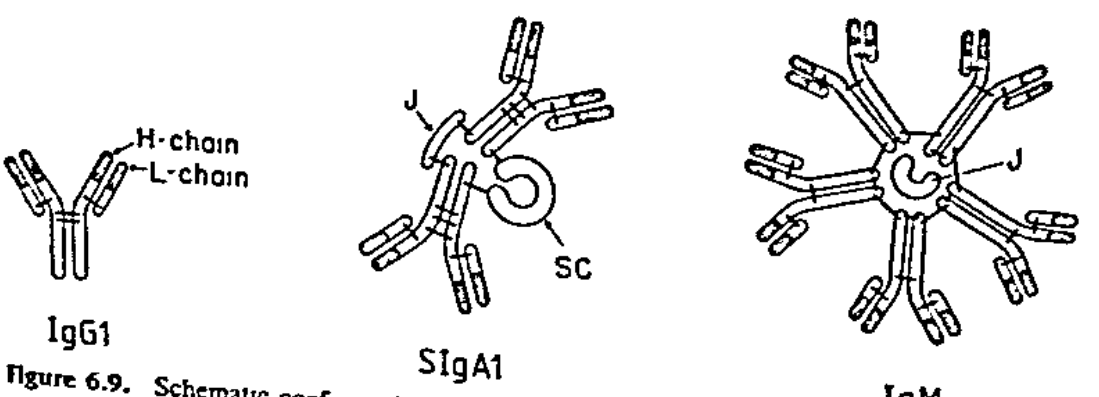



**Figure 6.9.** Schematic conformation of immunoglobulin IgG, SIgA, and IgM. ——— = disulfide linkage. Hatched = variable portion.

consisting of a single polypeptide chain with a number of internal disulfides. It has a relatively high content (10–15%) of bound carbohydrate consisting of N-acetylglucosamine, N-acetylgalactosamine, D-galactose, D-mannose, L-fucose, and N-acetylneuraminic acid. In addition to the amount that is bound in SIgA, it occurs in the free state (FSC) in milk in concentrations of 50–100 mg·liter$^{-1}$. (See Table 6.6.) Long before it was recognized as being a secretory component, the FSC had been isolated, partially characterized, and designated glycoprotein-a.

IgM consists of a pentamer of the basic four-chain units joined by the J-component. It has 11–12% bound carbohydrate and MW of 900,000; its diameter is about 30 nm. In all cases the sites that bind antibodies consist of the variable portion of an H and an L chain in juxtaposition to each other. IgG has two such sites, SIgA has four, and IgM has ten. The immunoglobulins of an animal thus consist of numerous different proteins; each antigen encountered has caused the synthesis of Ig with a different variable portion.

In bovine milk and colostrum the principal immunoglobulins are IgG1 and IgA, the former usually present in larger concentration. Calves are born without circulating antibodies, and the chief way in which they acquire them is by ingestion of colostrum. Typical concentrations of the several immunoglobulins in blood serum, colostrum, and milk are given in Table 6.6. The concentrations are highly variable, even in mature milk, and usually decrease with advancing lactation. Preparations of the immunoglobulin fraction of milk also include a very high molecular weight lipoprotein.

The immunoglobulins in milk can exert an antimicrobial action, particularly IgM, which acts as agglutinin, for instance, against some streptococci. (See Section 11.6.) Moreover, cow's milk (but not that of buffalo, goat, or sheep) contains a cryoglobulin (mainly consisting of IgM) that is involved in the cold agglutination of milk fat globules (Section 14.3) and in the attachment of bacteria to fat globules. The immunoglobulins are among the most heat-sensitive of the whey proteins.

**Table 6.6. Concentrations of Immunoglobulins in Bovine Blood Serum, Colostrum, and Milk**

| Immunoglobulin | g·kg$^{-1}$ | | | Percentage of Total Ig | | |
|---|---|---|---|---|---|---|
| | Serum | Colostrum | Milk | Serum | Colostrum | Milk |
| IgG1 | 11.00 | 47.60 | 0.60 | 50.0 | 81.0 | 73.0 |
| IgG2 | 7.90 | 2.90 | 0.02[a] | 36.0 | 5.0 | 2.5 |
| IgA | 0.50 | 3.90 | 0.14 | 2.0 | 7.0 | 18.0 |
| IgM | 2.60 | 4.20 | 0.05 | 12.0 | 7.0 | 6.5 |
| FSC | — | 0.20 | 0.05 | — | — | — |

[a]Some authors report IgG2 contents up to 10 times this amount.

### 6.4.5. $\beta_2$-Microglobulin

This protein consists of a single polypeptide chain of about 100 amino acid residues and MW of 11,800. It is present in several body fluids and in membranes of various types of cells. Its amino acid sequence indicates homology with the constant regions of immunoglobulin light and heavy chains.

A protein that had long previously been crystallized from bovine milk and designated lactollin was shown in 1977 to be bovine $\beta_2$-microglobulin. It is a polypeptide of 98 residues, two of which are Cys.

Direct analyses for microglobulin concentration have not been made in bovine milk; the amounts of lactollin that have been isolated from colostrum and milk are about 6 and 2 mg·liter$^{-1}$ respectively.

### 6.4.6. Lactoferrin and Transferrin

Two iron-binding proteins are found in milk. One of them, transferrin (Tf), is a common blood plasma protein; the other, lactoferrin (Lf), is secreted not only by mammary glands but also by lacrymal, bronchial, and salivary glands and by kidney and endometrial mucosa. It also is found in specific granules in heterophilic leukocytes. Both Tf and Lf appear to be large single-chain polypeptides of 600–700 residues. Reported molecular weights differ somewhat; recent work favors 75,000 to 77,000 for Tf, but values for Lf are not so consistent, either 77,000 or 93,000 being reported. In both proteins about 4 mol % of the residues are Cys, and both have covalently linked carbohydrate consisting of N-acetylglucosamine, mannose, galactose, and N-acetylneuraminic acid. All transferrins and lactoferrins yet worked with appear to bind 2 mol of $Fe^{3+}$ per mole. Tf and Lf differ markedly from each other in amino acid composition and in electrophoretic mobility. They can be detected readily in electrophoretic patterns by autoradiography with $^{59}Fe$. Electrophoretic patterns of milk and blood preparations from individual animals reveal the occurrence of genetic variants of both proteins. No immunological cross-reaction between Tf and Lf has been demonstrated even when both are from a single species. Amino acid analyses and partial sequences of human Lf and Tf indicate some degree of homology between the two and some internal homology of peptide segments within each; sequencing is far from complete, however.

Both Tf and Lf can be determined quantitatively in a biological fluid by immunodiffusion using a specific antiserum. The concentrations and ratios of Tf and Lf in milk vary greatly among species and with stage of lactation. The concentration of Lf in colostrum is about 1250 mg·kg$^{-1}$; in midlactation the concentration falls to less than 100 mg·kg$^{-1}$. Concentrations of Tf in milk have not been determined accurately but may be similar to those of Lf. Lactoferrin is an inhibitor of bacteria because it deprives them of iron. The concentration of Lf in bovine milk is so low, however, that it does not exert any significant antibacterial effect.

### 6.4.7. Proteose–Peptone Fraction

A fraction of the whey proteins amounting to about 1 g per kilogram of milk is not rendered acid-insoluble by previously heating the milk. It has long been called proteose–peptone. Four principal groups of components of the proteose–peptones are distinguishable electrophoretically. They are component 3, which is probably derived from a fat globule membrane constituent, and components 5, 8-fast, and 8-slow, which are derived by proteolysis of $\beta$-casein. (See Table 6.5.) Undoubtedly, there are a number of other constituents in the proteose–peptone in addition to those named.

### 6.4.8. Other Whey Proteins

A group of acid glycoproteins is retained on DEAE cellulose when blood serum or whey is passed through it at pH 4.5. Fractional elution separates a number of components. The total amounts that can be obtained from bovine blood serum, colostrum, and milk are, respectively, about 2.0, 1.0, and 0.3 g·liter$^{-1}$. One protein of this group is $\alpha_1$-acid glycoprotein, formerly called orosomucoid. It has been isolated from human serum, colostrum, and milk, and from bovine serum and colostrum, but it has not been detected in bovine milk. It consists of a polypeptide chain of 181 residues to which five heteropolysaccharide groups are linked to asparaginyl residues. The carbohydrate constitutes about 45% of the total molecule. The function of this protein is not known. In any event, $\alpha_1$-acid glycoprotein comprises only a small portion of the acid glycoproteins obtainable from fractionation of colostrum or milk on DEAE cellulose. Five other fractions have been obtained in varying states of homogeneity. All contain carbohydrate and phosphate and promote the growth of *Bifidobacterium bifidum* var. *pennsylvanicus* (formerly *Lactobacillus bifidus*). The possibility that some of these glycoproteins represent partial degradation products of caseins or membrane materials has not been elucidated.

A specific protein that binds folate (FBP) has been isolated from milk. Affinity chromatography on Sepharose to which folate has been attached is especially effective in isolating this protein. Its concentration in normal milk is about 8 mg·kg$^{-1}$.

## 6.5. FAT GLOBULE MEMBRANE PROTEINS

The fat globule membrane contains approximately 50% protein and accounts for about 1% of the total protein of the milk. Some of the protein constituents of the membrane are enzymes (Section 7.2), but it is not possible at present to estimate the ratio of enzymatic and nonenzymatic components. The fat globule membrane proteins are difficult to resolve analytically and to separate preparatively because they interact strongly with one another and with lipids,

and most of them are insoluble. Part is released from the globules by cooling the milk. Some success has been achieved in solubilizing the membrane proteins with detergents such as sodium dodecyl sulfate coupled with reduction of disulfides. Preparations solubilized in this way exhibit a great number of electrophoretically distinguishable components (as many as 40), some of which are revealed by specific stains to contain carbohydrate. Many attempts at fractionating the mixture have been made, and many analytical data on composition of various kinds of mixtures have been reported. Some preparations alleged to be electrophoretically homogeneous or nearly so have been obtained by ion exchange chromatography and gel filtration. Three such preparations isolated by different workers all were heavily glycosylated; they differed widely in amino acid composition. One membrane protein has been shown to be immunologically related to proteose–peptone component 3. It is clear that the mixture of fat globule membrane proteins is a long way from being resolved on a molecular basis.

## *Suggested Literature*

The structure of proteins is well described by

G. E. Schulz and R. H. Schirmer, *Principles of Protein Structure* (New York, Springer-Verlag, 1979).

A complete but somewhat unbalanced and now outdated coverage of milk proteins is given in this two-volume work:

H. A. McKenzie, ed., *Milk Proteins* (New York: Academic Press, 1970 and 1971).

Nomenclature and a summary of properties of milk proteins is in

W. N. Eigel et al., "Nomenclature of the Proteins of Cows' Milk," Fifth Revision, *J. Dairy Sci.* 67 (1984): XX–XX.

An up-to-date monograph on milk proteins is

P. F. Fox, ed., *Developments in Dairy Chemistry*, vol. 1· Proteins (London: Applied Science Publishers, 1982).

Immunoglobulins in lacteal secretions are reviewed by

J. E. Butler in *Lactation: A Comprehensive Treatise*, (B. L. Larson and V. R. Smith, eds.), vol. III, chap. 5 (New York: Academic Press, 1974)

# 7

# Enzymes

Approximately 50 enzymatic activities have been detected in bovine milk. Milk chemists are interested in quantitation of these enzymes, in deleterious or beneficial reactions that they catalyze in milk and dairy products, and in their inactivation. This chapter presents (1) an outline of basic enzyme kinetics as a basis for quantitation, (2) a summary of properties of some of the principal milk enzymes, and (3) a comparison of their susceptibility to heat inactivation.

## 7.1. KINETICS OF ENZYME-CATALYZED REACTIONS

Some enzymes catalyze in one minute the conversion of up to 1,000,000 molecules of reactant(s) to product(s) per molecule of enzyme; at the other extreme, other enzymes catalyze only a few hundred molecules of reactant(s) to product(s) per minute. The rate of catalysis per molecule of enzyme is expressed as $k_{cat}$, sometimes called the turnover number for the enzyme. The $k_{cat}$ is a function of the efficiency of the enzyme and the chemistry of the reaction. Frequently, neither $k_{cat}$ nor enzyme concentration is known, but the product of $k_{cat}$ and enzyme concentration can be measured and is expressed as quantity (micromoles) of product formed per unit time in a unit volume of sample. This value of quantity formed, when measured under appropriately defined conditions, is known as the maximum activity, $V_{max}$, of the enzyme. It is apparent that enzyme concentration is related to enzyme activity, $V_{max}$, through $k_{cat}$:

$$[E] = V_{max}/k_{cat} \tag{7.1}$$

It follows that activity can be used to compare the concentrations of two enzymes only when it is known that both are identical, that is, have the

same $k_{cat}$. Moreover, some enzymes may bind to substances present (e.g., casein micelles), thus lowering their activity.

Enzyme activity is frequently expressed in units. The Enzyme Commission has defined a unit of enzyme as the quantity that will catalyze the transformation of one micromole of substrate to product(s) per minute under standard conditions. Sometimes the activity of an enzyme preparation will be reported as specific activity, which is given as units per milligram of protein used in the assay. The investigator must take care when reporting enzyme activity that the value obtained truly measures $V_{max}$ and that standard conditions of temperature, pH, and ionic strength are defined. Precautons must be taken to avoid inactivating a portion of the enzyme or modifying its activity so that $k_{cat}$ is changed.

Although a linear relationship is assumed between enzyme concentration and velocity, deviations from linearity occur and must be taken into account. For instance, the velocity may diminish during the course of time required to measure the velocity of the reaction because of depletion of substrate (see below) or because one of the products is an inhibitor. For comparative purposes and quantitation, initial velocities are preferred. In order to employ the relation between reaction velocity and enzyme concentration to quantitate the latter, three other parameters—substrate concentration, temperature, and pH—must be recognized and controlled. In general a plot of initial velocity against substrate concentration is a section of a rectangular hyperbola. (See Figure 7.1A.) Such a relation is rationalized and explained on the basis of the well-known Michaelis–Menten equation:

$$v_i = V_{max}[S]/(K_m + [S]) \tag{7.2}$$

where $v_i$ = initial velocity of reaction, $V_{max}$ = maximum velocity (or activity), $K_m$ = Michaelis constant, and [S] = substrate concentration. This equation is derived on the basis of formation of a complex between enzyme E and substrate S with subsequent dissociation to enzyme and product P. Thus

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P \tag{7.3}$$

$K_m$ equals $\frac{(k_2 + k_3)}{k_1}$, and it may be demonstrated readily that $K_m$ is equal to the substrate concentration that gives half maximum velocity, or $K_m$ = [S] when $v_i = \frac{V_{max}}{2}$. It follows that when [S] = $K_m$ only half of the enzyme will be in the ES form, the remainder occurring without substrate bound to it. Evaluation of $K_m$ and $V_{max}$ from a direct plot of $v_i$ against [S], as in Figure 7.1A, lacks precision and various linear transforms have been employed to obtain more precise estimates of $K_m$ and $V_{max}$. Probably the most widely used transform is the Lineweaver–Burk plot of $\frac{1}{v_i}$ against $\frac{1}{[S]}$, in which the

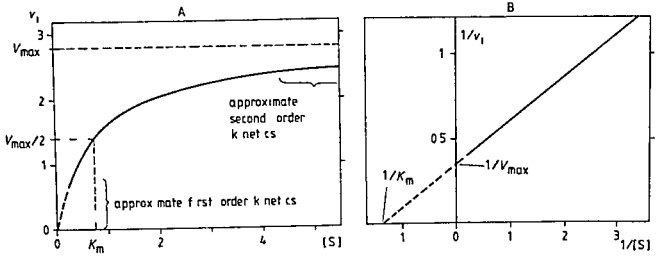



**Figure 7 1.** Effect of substrate concentration [S] on initial reaction rate $v_i$ assuming enzyme concentration to be constant The units are arbitrary B gives a Lineweaver–Burk plot of A

slope is $\frac{K_m}{V_{max}}$ and the intercepts give $\frac{1}{V_{max}}$ and $\frac{1}{K_m}$ (See Figure 7 1B ) $K_m$ is a measure of the affinity of an enzyme for its substrate, the lower the $K_m$, the greater the apparent affinity For many enzymes $K_m$ lies in the range of $10^{-3}$ to $10^{-6}$ $M$ Figure 7 1 shows that the kinetics of the reaction change from first order to zero order with respect to substrate concentration as the latter is increased Obviously, quantitation of enzyme activity preferably is made under conditions of zero-order kinetics with substrate in excess

Not all enzyme catalyzed reactions follow simple Michaelis–Menten kinetics For instance, allosteric enzymes (i e , enzyme molecules consisting of some protein subunits) frequently bind substrates at more than one site on the enzyme, and substrate binding at one site affects the affinity of that for another site The presence of inhibitors may affect the enzyme pattern Enzymes catalyzing multisubstrate reactions follow more complicated kinetic patterns, however, the same principle of substrate binding to enzyme following a hyperbolic binding relationship usually is obeyed

In general, enzymes are active only over a limited range of pH, and usually a distinct pH optimum is observed This may result from an effect of pH on the $V_{max}$, on affinity of enzyme for substrate, or on the stability of the enzyme The activity of many enzymes depends on particular ionized groups in the active site Obviously, these will be influenced by even small variations in pH and ionic strength More extreme high or low pH may denature and hence inactivate the enzyme

The overall effect of temperature on enzyme catalyzed reactions is a resultant of the accelerating influence of temperature on the reaction itself and the thermal denaturation and inactivation of the enzyme Thus, the rate of the catalyzed reaction passes through a maximum as it is examined at a series of increasing temperatures The optimal temperature, therefore, is generally higher when the time during which the reaction is measured is shorter The optimum differs greatly among individual enzymes because they differ in susceptibility to thermal denaturation (See Section 7 4 )

It should be apparent from this brief introduction that measurement of enzyme activity must be performed under rigidly controlled conditions in which observed activity can be translated into activity with 100% of the enzyme in the ES form. Therefore, enzyme activity is a value equivalent to $V_{max}$ for the concentration of enzyme available, that is, $[E]k_{cat}$. (The values given in Table 7.1 represent such values.)

## 7.2. SOURCE AND SIGNIFICANCE OF ENZYMES IN MILK

Enzymatic activities detected in bovine milk are listed in the appendix in Table A.10. They are named and classified according to the recommendations of the Enzyme Commission of the International Union of Biochemistry. All classes of enzymes except ligases have been detected in milk.

Some of the milk enzymes (e.g., catalase) are constituents of leukocytes, and some (e.g., plasmin) may gain entrance to milk from blood. Most, however, are constituents or products of the mammary cells that enter milk as rather benign accidental constituents during the secretory processes. Some (e.g., the galactosyl transferase component of lactose synthase) are constituents of Golgi membranes, others (e.g., alkaline phosphatase) of the cell membrane. Enzymes of microbial origin are not considered in this chapter. Individual milk enzymes are associated with casein micelles, fat globules, or leukocytes, or are dispersed in the serum. Locations are specified in the appendix, in Table A.10, for cases in which they are known.

Concentrations of milk enzymes vary greatly among species, and within a species large variations occur among individual animals and during the course of lactation. Data on such variations for most of the milk enzymes are not very complete or definitive.

A few of the enzymes found in milk (e.g., lactose synthase) have known functions in the biosynthetic processes of the mammary cells. Some milk enzymes act on substrates present as normal constituents of milk and may under suitable conditions play either beneficial or deleterious roles in dairy processes and products. Hydrolases, such as lipase and proteinase, may facilitate resorption of milk constituents if and when milking is stopped. There is no well-documented case of an enzyme in bovine milk being of direct benefit to the primary consumer—the calf. It has been suggested, however, that a lipase in human milk facilitates digestion of fat by the human infant.

## 7.3. SOME PROMINENT MILK ENZYMES

Activities of 14 important enzymes in bovine milk are listed in Table 7.1. By way of emphasizing the differences among species, ratios of activities in bovine and human milks are included. Xanthine oxidase, lactoperoxidase,

**Table 7.1. Activities of Some Enzymes in Bovine Milk**

| Enzyme | EC Number | Optimum pH | Optimum Temp (°C) | Activity[a] in Bovine Milk | Ratio of Activities Human/Bovine |
|---|---|---|---|---|---|
| Lactate dehydrogenase | 1 1 1 27 | | | 40 | 3 5 |
| Malate dehydrogenase | 1 1 1 37 | | | 40 | 1 8 |
| Xanthine oxidase | 1 2 3 2 | 6 –9 | 37 | 175 | — |
| Catalase | 1 11 1 6 | 7 0 | ? | 300 | 10 |
| Lactoperoxidase | 1 11 1 7 | 6 –7 | 20 | 22 000 | 0 03 |
| Superoxide dismutase | 1 15 1 1 | | | 0 1–2 5 | — |
| Galactosyl transferase | 2 4 1 38 | 7 2 | 37 | 50 | — |
| Lipoprotein lipase | 3 1 1 34 | | | 600 | — |
| Alkaline phosphatase | 3 1 3 1 | 9 8 | 37 | 500 | 0 02 |
| Phosphoprotein phosphatase | 3 1 3 16 | 4 0–5 5 | 14–50 | 13 | 1 2 |
| Ribonuclease | 3 1 27 5 | 7 5 | 37 | 11 0–25 0 mg[b] | 0 3 |
| Lysozyme | 3 2 1 17 | 7 9 | 37 | 130 μg[b] | 3000 |
| Fructose–bisphosphate aldolase | 4 1 2 13 | 7 | 35 | | 4 7 |
| Glucose phosphate isomerase | 5 3 1 9 | | | 155 | 2 |

[a] μmole $min^{-1}$ $liter^{-1}$ except as otherwise noted
[b] Mass per liter milk

and ribonuclease are especially prominent in bovine milk; lysozyme predominates quantitatively in human milk.

### 7.3.1. Oxidases

*Xanthine oxidase* (XO, EC 1.2.3.2) is very prominent in bovine milk. Most of it is associated with the fat globule membrane. Because of its relatively high XO content, milk is a source of choice for isolating this enzyme for investigation. Xanthine oxidase is much less prominent in milks of most other species; goat's milk has only about one-tenth the activity of cow's milk. The activity of XO in cow's milk depends to some extent on the Mo content of the feed consumed.

The molecular weight of xanthine oxidase is about 283,000; it is a dimer of two identical subunits, each carrying one flavin-adenine dinucleotide, one Mo, four Fe, and four acid-labile S atoms. The specific activity of the pure enzyme is 5 $\mu$mol·min$^{-1}$·mg$^{-1}$. It can oxidize 12 moles of xanthine per mole per second (assuming two active centers per dimer). Although hypoxanthine and xanthine are its normal substrates in the pathway of purine metabolism, it catalyzes oxidation of many other substrates, including various aldehydes. Under some conditions XO catalyzes a two-electron reduction of $O_2$ to $H_2O_2$; but at high pH, high $O_2$ concentration, and low xanthine concentration, the one-electron reduction to superoxide ion, $O_2^-$ is favored.

As isolated from milk, XO utilizes molecular $O_2$ as an electron acceptor, but if some of its disulfides are reduced with dithiothreitol it becomes a $NAD^+$-dependent dehydrogenase. Reoxidizing the thiols to disulfides with the milk enzyme sulfhydryl oxidase (see below) restores the specificity of the enzyme for $O_2$ as electron acceptor.

The activity of XO in fresh milk is increased about fourfold by storing at 4°C, by heating at 70°C for 5 min, by homogenization, or by incubation with commercial proteinase or lipase preparations. Such treatments also transfer much of the enzyme from fat globules to plasma. It becomes more sensitive to inactivation by heat treatment with increasing time of storage at 4°C. The XO activity (fully activated) of milk is about 175 $\mu$mol $O_2$·min$^{-1}$·liter$^{-1}$. The specific activity of the enzyme is 5 $\mu$mol·min$^{-1}$·mg$^{-1}$; its concentration in milk, therefore, is about 35 mg·liter$^{-1}$. (Older reports of higher concentrations were based on calculations from the specific activity of less highly purified preparations.) Considerable study of the possibility that XO is involved in the development of oxidized flavors in milk has not yet led to definite results. XO can reduce $NO_3^-$ (which occurs in milk only in trace quantities) to $NO_2^-$, which is a powerful inhibitor of some bacteria. This property is put to use in the manufacture of certain types of cheese, where a little nitrate is added to the milk to prevent late blowing of the cheese by Clostridia.

A *sulfhydryl oxidase* (EC number not assigned), present in bovine milk, catalyzes oxidation of thiols to disulfides using molecular $O_2$ as the electron

acceptor It is an aerobic oxidase, reducing the $O_2$ to $H_2O_2$ rather than to $H_2O$ Thus

$$2\,RSH + O_2 \rightarrow RSSR + H_2O_2 \qquad (7\,4)$$

(It is completely different from thiol oxidase—EC 1 8 3 2—not known to occur in milk, which forms $H_2O$ in the oxidation ) Sulfhydryl oxidase catalyzes oxidation of thiols in both small compounds and proteins It is a large aggregate of subunits of MW 89,000 It contains about 11% carbohydrate residues by weight and 0 5 atom Fe per subunit At optimal pH (7 0) and temperature (35°C) its $K_m$ for glutathione as substrate is 90 $\mu M$ Preparations with specific activities of more than 50 $\mu$mol $min^{-1}$ $mg^{-1}$ have been obtained Milk may contain about 3 mg of this enzyme per liter Sulfhydryl oxidase is closely associated with $\gamma$-glutamyl transferase (EC 2 3 2 2) in skim milk, and it has been suggested that the two activities reside in the same molecule Such suggestions have been proved wrong, however, because the two activities can be separated Sulfhydryl oxidase immobilized on glass beads has been used to oxidize thiols in UHT milk in an attempt to reduce cooked flavor

*Catalase* (EC 1 11 1 6) catalyzes the decomposition of $H_2O_2$ to $H_2O$ and $O_2$ It is found in many tissues and is particularly prominent in liver, erythrocytes, and kidneys of animals In milk its activity parallels leukocyte count and is higher in mastitic milk and colostrum than in normal milk It increases with the multiplication of bacteria Cells associate with the fat globules therefore, catalase accumulates in the cream layer Crystalline catalases have been prepared from several sources, but because little is present in milk it has not been fully purified therefrom Milk catalase has a molecular weight of about 210,000, its isoelectric pH $\approx$ 5 5, and it contains heme iron Catalase activities on the order of 300 $\mu$mol $min^{-1}$ liter $^{-1}$ have been reported for milk (See Table 7 1 )

*Lactoperoxidase* (LP, EC 1 11 1 7) catalyzes oxidation by $H_2O_2$ of a long list of electron-donor compounds, including aromatic amines, phenols, aromatic acids, leuko dyes, tryosine and tryptophan, ascorbate, iodide, nitrite, and thiocyanate LP may amount to as much as 1% of the total serum proteins of milk (i e , 60 mg $kg^{-1}$) Its activity in milk increases with advancing lactation to a maximum about 40 days postpartum and thereafter declines somewhat It is a glycoprotein (MW = 77,500) containing 20–26 hexosamine residues (no sialic acid) and one heme per mole Whether it consists of one or two polypeptide chains has not been settled LP catalyzes oxidation of thiocyanate ($SCN^-$) to a product ($OSCN^-$?) that inhibits certain bacteria Thiocyanate is a natural constituent of milk (see appendix Table A 9) and $H_2O_2$ is produced by some bacteria themselves Thus, in milk such bacteria exhibit self inhibition (See Section 11 6 )

*Superoxide dismutase* (SOD, EC 1 15 1 1) catalyzes the dismutation of superoxide ion $O_2^-$ to $H_2O_2$ and $O_2$ The enzyme consists of two identical

subunits of MW 16,000, each containing one Cu and one Zn per mole. The primary structure of SOD from bovine erythrocytes is known completely. It has one free thiol and one disulfide bond. It functions as a noncovalently linked dimer, and its specific activity is 3300 $\mu$mol·min$^{-1}$·mg$^{-1}$. Bovine milk serum contains a superoxide dismutase that is similar if indeed not identical to that of the erythrocytes. Estimates of the content in milk range from 0.15–2.4 mg·kg$^{-1}$. It is a rather heat-stable enzyme; the activity is not decreased by heating milk at 63°C for 30 min. It may well be an important antioxidant protecting milk constituents from oxidation by superoxide ion generated by oxidations catalyzed by xanthine oxidase and lactoperoxidase as well as by riboflavin-sensitized photoreactions.

### 7.3.2. Transferases

The only transferase discussed here is the galactosyl transferase component of *lactose synthase* (EC 2.4.1.22). In the absence of $\alpha$-lactalbumin this enzyme transfers a galactosyl residue from UDP-galactose to an N-acetylglucosamine residue either free or in a protein-bound oligomer. In performing this function the enzyme is sometimes designated glycoprotein $\beta$-D-galactosyl-transferase (EC 2.4.1.38). As pointed out previously (Section 6.4.1), $\alpha$-lactalbumin so modifies the transferase that the $K_m$ for transfer of a galactosyl group to D-glucose is much reduced, and a $\beta$-1,4-glycosidic linkage is formed. The transferase is found in membranes of Golgi vesicles and there performs the synthesis of lactose as $\alpha$-lactalbumin transiently combines with it. It enters milk with Golgi membrane fragments. Its concentration in bovine milk is approximately 3–4 mg·liter$^{-1}$. The specific activity of the purified enzyme is 14 $\mu$mol·min$^{-1}$·mg$^{-1}$ at 37°C and neutral pH. The activity in milk is, then, 40–60 $\mu$mol·min$^{-1}$·liter$^{-1}$. As isolated from milk, the transferase has a molecular weight of about 42,000. Higher values have been found for the enzyme isolated from tissue or from colostrum. Trypsin or plasmin cleave the molecule to the 42,000 size and the action of plasmin after secretion is probably responsible for finding that size in milk. The transferase contains 12–13% carbohydrate consisting of about 8% neutral sugars, 1% glucosamine, 1% galactosamine, and 2% sialic acid.

### 7.3.3. Hydrolases

The principal lipolytic enzyme of bovine milk is a *lipoprotein lipase* (EC 3.1.1.34). The normal function of such enzymes is to liberate fatty acids from lipoproteins and chylomicrons of the blood; the fatty acids are then resorbed by the secretory cells of the mammary gland. A low molecular weight apoprotein cofactor is necessary for the enzyme to attack its acyl glycerol substrate when the latter is present as or covered with lipoproteins. Blood serum contains such cofactors, and the lipoprotein lipase is said to be serum-stimulated. In bovine milk the lipoprotein lipase (about 2 mg liter$^{-1}$)

is bound largely to casein micelles. It does not attack the glycerides of the fat globules unless the membranes of the latter are damaged (e.g., as by homogenization) or if lipoproteins containing the apoprotein cofactor are added. The latter effect may arise from leakage of blood serum into milk if the tight junctions between secretory cells are impaired. (See also Section 5.5.) This enzyme is inactivated at pH 4.6 for 1 h. Lipoprotein lipase has been isolated from bovine skim milk using affinity chromatography on Sepharose to which heparin has been linked covalently. Its molecular weight is about 50,000, and it exists as a noncovalently linked dimer under physiological conditions; it contains about 8% carbohydrate. Preparations with activities capable of releasing 300–700 μmol of fatty acids (from a triglyceride emulsion at pH 8.5) per minute per milligram have been achieved.

Cow's colostrum contains little of the lipoprotein lipase but has a different lipase that is not bound to casein, does not bind to heparin–Sepharose, is not activated by blood serum, and is stable at pH 4.6 for 1 h. It disappears after the first few milkings following calving. The two lipases do not exhibit immunological cross-reaction. The colostral lipase probably falls under the classification of a triacylglycerol lipase (EC 3.1.1.3) but does not appear to be homologous to the bile-salt stimulated lipase of human milk. Lipolytic activities of skim milk and colostrum are respectively about 600 and 200 $\mu mol \cdot min^{-1} \cdot liter^{-1}$ (measured with tributyrin emulsion at pH 8.75, 37°C).

Milk *alkaline phosphatase* (EC 3.1.3.1) is a phosphomonoesterase. Two major isozymes have been identified, $\alpha$ and $\beta$ phosphatase, mainly located in the milk plasma and fat globule membrane, respectively. The latter, more abundant, isozyme has been highly purified from bovine milk and found to be a dimer of two identical or very similar subunits each of MW ~ 85,000. It contains about five atoms of zinc per dimeric molecule. Its optimal pH for hydrolysis varies from one phosphate ester to another and with the composition of the medium. Monoesters, such as phosphoserine and $\beta$-glycerophosphate are hydrolyzed maximally near pH 9.

A second phosphatase present in milk has a pH optimum at about 4.0. It is probably the *acid phosphomonoesterase* (EC 3.1.3.2), though it also has been assumed to be more similar to the phosphoprotein phosphatase (EC 3.1.3.16) of bovine spleen. It is primarily in the milk plasma. Its concentration is quite low (compared to alkaline phosphatase), though higher in colostrum.

Both the alkaline and the acid phosphatase can release inorganic phosphate from caseins and from soluble esters, and this may occur in milk or fractions thereof under appropriate conditions. The acid phosphatase is the more active of the two at the pH of milk. These two enzymes differ greatly in susceptibility to inactivation by heat. (See Section 7.4.)

The *ribonuclease* (RNase, EC 3.1.27.5) content of mixed milk was found in one study to be 11 $mg \cdot liter^{-1}$ and 25 $mg \cdot liter^{-1}$ in another. RNase has been isolated from bovine milk by various methods; it appears to be identical to bovine pancreatic RNase in amino acid composition and immunological cross-reaction. Bovine pancreatic RNase has been well characterized. It

consists of a single polypeptide chain of 124 residues, with MW 13,690. In spite of the low content of RNase in human milk (3 mg·liter$^{-1}$), it has been isolated from that source by adsorption on a cation exchanger. Both RNase and lysozyme are so adsorbed; they can be differentially eluted.

*Lysozyme* (EC 3.2.1.24) is quantitatively an important fraction of the whey proteins of human milk (400 mg·liter$^{-1}$). It is a powerful bactericide as it attacks polysaccharides of the bacterial cell wall, causing lysis of the bacteria. Bovine milk contains only about 0.1 mg·liter$^{-1}$ of lysozyme; nevertheless, lysozyme has been isolated from it. The lysozyme of human milk appears to be identical to that found in other human secretions; it is a polypeptide of 129 residues, with MW 14,602. $\alpha$-lactalbumin and lysozyme are considered to be descendants of a common ancestor. Human lysozyme and $\alpha$-lactalbumin, both obtained from milk, have different residues at 81 of 129 positions. Both have four disulfide bridges identically placed.

The principal milk *proteinase* belongs to the alkaline serine proteinase class; it is probably identical to the plasmin of blood (EC 3.4.21.7). Blood plasminogen (human) is a polypeptide of 790 residues; activation involves proteolytic cleavage of the C-terminal 230 residues and sometimes the N-terminal 76 residues as well. Apparently, this enzyme enters milk from blood mostly in the form of its zymogen, plasminogen. In fresh milk only a small proportion is in the active form; milk may contain a factor that slowly activates the plasminogen. The enzyme is associated with the casein micelles. It attacks peptide bonds at the C-terminal side of Arg and Lys residues and thus is trypsin-like. Cleavage of Lys-X is faster than that of Arg-X. Optimal activity occurs at slightly alkaline pH and 37°C. The milk proteins most susceptible to plasmin are $\beta$- and $\alpha_{s2}$-caseins. $\alpha_{s1}$-casein also is attacked while $\kappa$-casein is relatively resistant, and the whey proteins $\alpha$-lactalbumin and $\beta$-lactoglobulin are not affected. Plasmin action on $\beta$-casein is responsible for production of $\gamma$-caseins and the proteose–peptone fragments. (See Section 6.3.4.) Plasmin fully survives pasteurization and partially resists UHT treatments. Increased activity has even been observed after heating milk at 72°C for 15 s. This has been attributed to conversion of plasminogen to plasmin, to inactivation of inhibitors, or to enhancing the accessibility of susceptible linkages in the substrate. The action of plasmin may produce serious defects in UHT milk products, such as development of a bitter flavor (caused by hydrophobic peptides of low molecular weight) and changes in viscosity and appearance.

A second proteinase, with maximal activity at pH 4.0, also occurs in milk. Its molecular weight is 36,000, it is heat-labile (inactivated at 70°C for 10 min), and it is partly inhibited by SH-blocking agents. It cleaves $\alpha_{s1}$-casein faster than $\beta$- or $\gamma$-caseins (at pH 5, 37°C). Its action on these proteins produces peptides that in electrophoretic behavior resemble those produced by chymosin. It is not yet clear how this enzyme should be classified; it may well be an aspartate proteinase.

## 7.4. INACTIVATION

Enzymes usually are inactivated by all conditions that cause denaturation of globular proteins (See Section 6 1 ) They also can be inactivated by intense ionizing radiation and by prolonged shearing of the solution (at least some enzymes) Most interesting is inactivation by heat treatment, which is mostly irreversible It usually depends closely on conditions like pH, ionic strength, and water activity (See Section 17 2 for the latter )

The kinetics of heat induced reactions in milk are discussed in Section 10 2, and the theoretical basis for the widely used plots of log time versus temperature for a given amount of reaction is presented there Such plots for the inactivation of various enzymes in milk are given in Figure 7 2 They should be interpreted with care, small changes in the environment may alter the curves For instance, some enzymes are more stable in the presence of substrate than in its absence Moreover, the different isozymes of one enzyme (if present) may show a slightly different relation, often causing curved

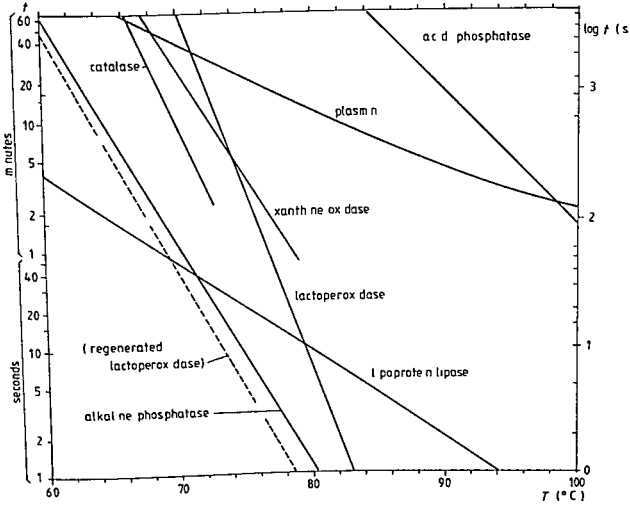



**Figure 7 2** Time ($t$) during which milk must be heated at a certain temperature ($T$) to inactivate some enzymes If known the curve is drawn for 99% inactivation (two decimal reductions) After various sources

plots. Some bacterial lipases even exhibit a distinct break in the log time versus temperature plot. Figure 7.2 shows a remarkable range of stability. The difference between the two phosphatases is noteworthy. Alkaline phosphatase has long been employed as an index of adequate pasteurization because it is inactivated by treatments similar to those required to kill tuberculosis bacteria. Certain other enzymes sometimes have been suggested as indicators of adequacy of heat treatments for other dairy products (especially low-heat and high-heat dry products), but no test of this kind has gained wide acceptance, though a lactoperoxidase test is used in some countries.

Certain enzymes exhibit some reactivation after high-temperature, short-time heat treatments. This means that a small amount of activity is regained during storage after the heat treatment. This effect is especially noticeable with alkaline phosphatase and with the natural milk proteinase–plasmin. The reactivation of alkaline phosphatase, which is chiefly observed in cream (because of its high phosphatase content), may cause problems in the control of pasteurization; it is uncertain from the phosphatase test whether or not the product is inadequately pasteurized. It appears that only one of the isozymes (the one exclusively found in the fat globule membrane) is reactivated. Lactoperoxidase also shows a slight and fairly rapid reactivation; the reactivated enzyme is again heat-labile, even more than the original enzyme. This complicates the determination of the inactivation curve.

## Suggested Literature

A rather complete one-volume work is

M. Dixon and E. C. Webb, *Enzymes*, 3rd ed. (New York: Academic Press, 1979).

The chemistry of many individual enzymes is dealt with exhastively in this 13-volume work edited by P. D. Boyer

*The Enzymes*, vols. 1–13 (New York: Academic Press, 1970–76).

A review of enzymes in bovine milk is

K. Shahani, W. J. Harper, R. G. Jensen, R. M. Parry, and C. A Zittle, *Enzymes in Bovine Milk: A Review*, *J. Dairy Sci.* 56 (1973). 531

Some of the important enzymes in milk are discussed in some detail by

M. L. Groves in *Milk Proteins*, H. A. McKenzie, ed., vol II, chap. 16 (New York: Academic Press, 1971).

And more recently by

P. F. Fox and P. A. Morissey in *Enzymes in Food Processing*, G. G Birch, N. Blakebrough, and K. J. Parker, eds. (London Applied Science, 1981).

# 8

# Miscellaneous Components

This chapter deals with a large number of milk components that are present in low concentration (generally less than 100 mg kg $^{-1}$), most of them do not fit unequivocally into any of the classifications in the previous five chapters They are grouped into the three categories of natural components, contaminants, and radionuclides

## 8.1. NATURAL COMPONENTS

The compounds included in this section appear to be natural in that they are present in freshly drawn milk and have been detected in all or nearly all samples in which they have been sought Compounds that have gained access to milk either accidentally or through chemical or bacterial action after milking are not included in this section Many of these natural components are intermediate products of the metabolic processes that occur in the mammary gland For convenience they are grouped as gases, trace elements, alcohols, aldehydes, ketones, and carboxylic acids, nonprotein nitrogenous compounds, sulfur-containing compounds, conjugated compounds, phosphate esters, nucleotides and nucleic acids, and hormones

Minor lipids are discussed in Sections 5 1 and 5 2 (Tables A 3 and A 4 in the appendix), enzymes in Chapter 7 (Table A 10 in the appendix), and vitamins in Section 19 6 (Table A 14) Minor carbohydrates are discussed in Section 3 4 or are included in one of the other categories, notably phosphate esters Some components are listed in more than one category A total of 171 compounds is considered in this section, not including trace elements

Improvements in the sensitivity and specificity of analytical methods have resulted in a great increase in the number of compounds detected in milk. Many of the components mentioned below have been detected by qualitative tests but not quantitated. Widely differing contents have been reported for some of those that have been quantitated; data are insufficient to date to enable us to decide whether such differences are caused by natural or analytical variation.

### 8.1.1. Gases

The gases $CO_2$, $N_2$, and $O_2$ are present in anaerobically drawn milk in concentrations of about 6.0%, 1.0%, and 0.1% by volume respectively. $CO_2$ is, of course, in equilibrium with bicarbonate ion. (See Section 4.2.) Its concentration falls rapidly in milk exposed to the air and virtually the entire $CO_2$—$HCO_3^-$ system can be removed by heating or vacuum treatment. The concentration of bicarbonate in mixed raw milk is generally 2 mmol·kg$^{-1}$ (Refer back to Table 4.1.) Both the $O_2$ and $N_2$ contents increase upon exposure of milk to air. Raw commercial milk contains at room temperature about 1.3% $N_2$ and 0.5% $O_2$ by volume, or about 15 and 6 mg·kg$^{-1}$, respectively.

### 8.1.2. Trace Elements

The elements listed in Table A.1 in the appendix have been detected in normal milk by spectrometric and chemical methods. Data on concentration and form of occurrence are rather scanty although Mo appears to be exclusively in xanthine oxidase and Co exclusively in vitamin $B_{12}$. Iron is an essential component of xanthine oxidase, lactoperoxidase, and catalase, and it is needed for the action of sulfhydryl oxidase. The trace metal present in highest concentration is Zn (~ 3 mg·kg$^{-1}$); about 85% of Zn is associated with the casein micelles (predominantely as part of the colloidal phosphate) and perhaps a small percentage with the fat globules. Cu has been studied extensively in regard to its importance to autoxidation of milk lipids. The natural Cu content of milk is about 20 μg·kg$^{-1}$, about 10% of which is in the fat globule membranes; colostrum may contain more than 100 μg·kg$^{-1}$. Milk contains about 200 μg·kg$^{-1}$ Fe, of which nearly half is in the membranes. Mn (~ 20 μg·kg$^{-1}$) is of importance in the metabolism of some lactic acid bacteria, notably for their fermentation of citrate, and in some milks the Mn content is too low for production of diacetyl. Fe and Mn do not catalyze lipid oxidation in milk products. The nutritional significance of some trace elements is outlined in Table A.1.

Trace elements may enter milk either through the cow or by contamination subsequent to milking. Concentrations of some of these elements in milk (e.g., B, Br, Co, I, Mn, Mo, Se, Zn) can be increased by increasing their level in the ration of the cow. Milk of cows raised in the vicinity of certain industrial plants may have relatively high levels of Cd or Pb; cows raised

close to highways tend to produce milk with high Pb content Contact of milk with metal containers and equipment is an important source of contamination with Cu, Fe, Ni and Sn

### 8.1.3. Alcohols, Aldehydes, Ketones, Carboxylic Acids, and Esters

Ethanol and the carbonyl and nonamino aliphatic carboxylic acid compounds listed in Table A 11 in the appendix have been detected in the free state in fresh milk, that is to say that they are not linked covalently to other compounds Some of them have not been detected in all samples in which they were sought Derivatization with 2,4-dinitrophenyl hydrazine and various techniques of volatilization, extraction, and chromatography have been valuable in detecting, separating, and quantitating these compounds

The acids, of course, are ionized at the pH of milk, and some of them are associated ionically with Ca Citric acid is included to make the list of carboxylic acids complete It is dealt with in more detail as a major salt constituent in Chapter 4 The concentration of citrate in milk is about 10 mmol $kg^{-1}$, that of all of the other carboxylic acids together probably totals 1–3 mmol $kg^{-1}$ in most milks Bacterial action may increase considerably the concentration of some carboxylic acids, notably lactic, acetic, and pyruvic acids, while part of the citric acid may be metabolized Lipolysis causes an increase in free fatty acids (See Section 5 5 )

In addition to the compounds mentioned in Table A 11 in the appendix, several others have been detected in heated milk This is discussed in Chapter 18, see particularly Figure 18 3

### 8.1.4. Nonprotein Nitrogenous Compounds

Compounds included here are of low molecular weight and are not precipitated with the proteins by 12% trichloroacetic acid Most of them are also dialyzable, but no systematic comparison of compounds present in trichloroacetic acid filtrates with those in dialysates seems to have been made Recent analyses show that some peptides of low molecular weight are present in the 12% trichloroacetic acid filtrate

The nonprotein nitrogen (NPN) content of milk is 250–350 mg $kg^{-1}$ or about 6% of total nitrogen, its total mass is about 1 g $kg^{-1}$ The principal components, accounting for most of the NPN, are listed in Table 8 1 Analyses in the literature differ considerably in concentrations reported for these constituents, and the range is undoubtedly wider than indicated in the Table This is because most NPN compounds are metabolites of amino acids and nucleic acids, and their concentration in milk thus greatly depends on the protein content of the ration Other nitrogen containing compounds, some of which have been detected but not quantitated, are compiled in the appendix in Table A 9

**Table 8.1. Principal Nonprotein Nitrogenous Compounds in Milk**

| | Recent Report[a] | Range in Literature |
|---|---|---|
| Urea-N | 142 | 84–280 |
| Creatine-N | 25 | 6–20 |
| Creatinine-N | 12 | 2–9 |
| Uric acid-N | 8 | 5–8 |
| Orotic acid-N | 15 | 12–13 |
| Hippuric acid-N | 4 | 4 |
| Peptide-N | 32 | |
| Ammonia-N | 9 | 3–14 |
| α-amino-acid-N | 44 | 29–51 |
| Total NPN | 296 | 229–308 |

*Note:* Contents in mg per kg milk.
[a]H. Wolfschoon-Pombo and H. Klostermeyer, Milchwissenschaft 36 (1981).598.

Urea accounts for about half of the NPN of milk; it is one of the few miscellaneous milk constituents whose concentration exceeds 100 $mg \cdot kg^{-1}$; it is very variable (200–600 $mg \cdot kg^{-1}$). The amino acids contributing to the α-amino N include most of those that are also present in proteins plus a few additional ones; free cysteine appears to be absent. Little is known at present about the identity of the constituents of the peptide category.

Bovine milk contains 1–2 $g \cdot kg^{-1}$ of oligosaccharides; colostrum has considerably more. To be sure, these are predominantly bound to proteins, notably immunoglobulins, κ-casein, and fat globule membrane proteins. The five oligosaccharides that have been isolated from colostrum all contain nitrogen as N-acetyl or N-glycolyl neuraminic acid, and one of them has N-acetyl glucosamine unit as well. Small amounts of free N-acetyl neuraminic acid and N-acetyl glucosamine also occur in milk. Orotic acid is particularly a hallmark of the milks of ruminant animals; milks of other species have little if any of this constituent.

### 8.1.5. Sulfur-Containing Compounds

Sulfur occurs in several states of oxidation in compounds in milk. Methyl sulfide, $(CH_3)_2S$, has been implicated as the constituent primarily responsible for "cowy" flavor in milk. (See Section 18.2.) The concentration of this material in milk is in the range of 10–40 $mg \cdot kg^{-1}$; feeding fresh alfalfa (lucerne) pushes the concentration to the higher part of the range. Dimethyl sulfone, $(CH_3)_2SO_2$, in concentrations of 6–8 $mg \cdot kg^{-1}$, has been detected in milk. Indoxyl sulfate has been found in milk in concentrations of 0.3–2.2

mg·kg$^{-1}$. (See appendix, Table A.9.) Lipoic acid, an important carrier of acetyl groups in metabolic reactions, has been identified in milk, but its concentration seems not to have been determined.

Taurine concentration in milk is about 4 mg·kg$^{-1}$; bovine colostrum and milks of other species, especially human milk, contain much higher concentrations of taurine. (See appendix, Table A.9.) The possible nutritional significance of this compound is discussed in Chapter 19. Thiocyanate, $SCN^-$, has been reported frequently as a constituent of normal milk. There are large variations in the content reported both within and among the results of various workers. These may be related to season and feed. Many of the analyses fall in the range of 1–5 mg·kg$^{-1}$, but some are much higher, up to 20 mg·kg$^{-1}$.

### 8.1.6. Conjugated Compounds

Many compounds apparently conjugated with glucuronate or sulfate have been detected in milk. The conjugates are adsorbed from milk on a neutral resin, eluted and hydrolyzed with glucuronidase and aryl sulfatase. In one study some 42 such compounds were identified. (See Table A.13 in the appendix.) Several of them also occur in the free state. (See Tables A.9 and A.11.) It is not known which are conjugated with glucuronate and which with sulfate, and curiously enough glucuronate itself has not been detected in milk in either bound or free state.

### *8.1.7. Phosphate Esters*

Milk contains about 100 mg·kg$^{-1}$ of phosphorus in the form of soluble organic compounds. Many of these are esters of orthophosphoric acid. A list of those that have been detected is in Table A.2 in the appendix. They are mostly sugar phosphates and constituents of phospholipids.

### 8.1.8. Nucleotides and Nucleic Acids

The list of nucleotides detected in milk has grown rapidly as more sensitive methods have been developed. (See appendix, Table A.12.) The common mono- and di-nucleotides, 3′,5′cyclic-AMP, and ATP are all present. ATP is located entirely in the casein micelles. Several nucleotide sugars, doubtless intermediates in glycoprotein synthesis, are found in milk. Both RNA and DNA have been detected and quantitated; leukocytes probably are an important source.

### 8.1.9. Hormones

The protein hormone prolactin, of 199 amino acid residues and MW 22, 663, is normally present in milk at a concentration of about 50 μg·kg$^{-1}$. Prolactin secreted in bovine milk has been shown to be biologically active in stimu-

lating the secretory activity of rabbit mammary cells. Milk may contain placental lactogen and luteotropin, but, if so, the concentrations are much smaller than those of prolactin.

The hypothalamic hexapeptide gonadotropin-releasing hormone (GnRH) has been found in milk in concentrations of 1.5 $\mu g \cdot kg^{-1}$, and a second hypothalamic peptide, thyrotropin-releasing hormone (TRH) may be present in much lower concentration.

There are no clearcut demonstrations of the presence of any of the amine hormones of the adrenal medulla (e.g., adrenaline) or of the thyroid (e.g., thyroxin) in bovine milk, although the latter has been found in human milk.

Steroid hormones, both from the adrenal cortex and from the ovary, are present in milk. In the former category the glucocorticoids cortisol and corticosterone total 0.2–0.6 $\mu g \cdot kg^{-1}$. Progesterone concentration is about 10 (up to 30), estrone 28, and estradiol (mostly 17 $\alpha$- but a little 17 $\beta$-) about 175 $\mu g \cdot kg^{-1}$. Metabolic products of the steroids—5$\alpha$-androstane-3,17-dione and 5$\alpha$-pregnane-3,20-dione-also have been detected in small concentrations.

## 8.2. CONTAMINANTS

Milk and milk products may contain numerous components in very small concentrations (say $\mu g \cdot kg^{-1}$), that are called contaminants. It is difficult to distinguish these from natural milk components. We may define contaminants as chemical substances that do not occur in the milk of cows kept under natural conditions (although natural conditions are hard to define). Contaminants primarily include manmade chemicals. Some of these also occur in nature (e.g., all trace elements), and in such a case contamination may be a matter of concentration or route of entrance in the milk rather than presence. Natural substances, like toxins of microbial origin and endogenous plant toxicants, may occur in the cow's feed and may gain entrance to the milk; then they would commonly be called contaminants.

Of greatest concern are substances that are potentially harmful to the consumer's health. This may concern *toxic* compounds; examples are various pesticides. In view of the usually minute concentrations, acute toxicity is very rare for milk contaminants, and it usually concerns chronic toxicity. Substances may be mutagenic (i.e., cause gene damage), hence potentially *carcinogenic* (e.g., aflatoxins.) Other compounds cause *allergy* in some people (e.g., penicillin); natural milk also contains allergens (mostly proteins), though they affect very few people. Suspicions that consumption of milk that contains antibiotics may induce a personal microflora resistant to those antibiotics have not been confirmed.

In view of potential hazards to the consumer, public health authorities of many countries have set concentration limits for numerous contaminants in milk products (and other foods). It is never the presence of any substance that is important (it depends moreover on the sensitivity of the analytical

method employed), but the level. The limits are thus based on the maximum dose of the substance that is generally considered safe, which varies widely (by several orders of magnitude) among substances, and on the quantity of the product usually consumed. Such limits include a wide margin because of differences among people in susceptibility to a certain toxicant and in eating habits.

Contaminants may be detrimental to the food quality of the milk or its derivatives. Several substances impart off-flavors, which are further discussed in Section 18.3.1. Others may induce chemical reactions causing off-flavors; a notorious example is Cu and fat autoxidation. Some substances interfere with manufacturing processes; for example, antibiotics and disinfectants inhibit bacterial action, hence fermentation of milk. Finally, milk may be expected to contain several contaminants that are not harmful or detrimental and, therefore, often go unnoticed.

There are several pathways by which contaminants gain entrance into the milk:

1. Substances can be excreted by the glandular epithelium of the udder, which implies that they are in the blood. The common way of uptake by the cow is through the digestive tract; this concerns mainly contaminants of the feed and possibly drugs administered. Another route is through the air (e.g., volatiles, aerosols). Finally, drugs or other medicaments can be taken up through the skin or directly injected. The concentration of a contaminant may be much diminished by passage through the cow: substances may be broken down in the digestive tract; they may be absorbed poorly or not at all by the intestinal mucosa and be excreted with the feces; they may take part in the metabolism and be broken down, for instance, in the liver; they may be excreted by way of the urinary tract, the lung, or the skin; and, finally, they may be excreted into the milk, though the glandular epithelium constitutes a barrier of some resistance. The cow thus acts as a filter but, as each compound has its own fate, of widely variable effectiveness. It will be clear that, besides the substance itself, metabolites of a contaminant may enter the milk; well-known examples are DDE (metabolite of DDT) and aflatoxin $M_1$ (metabolite of aflatoxin $B_1$).

   Some compounds tend to accumulate in the body and can thus be excreted into the milk long after uptake by the cow. For instance, several di- and trivalent cations are deposited in the bone. A notorious example is the accumulation of lipophilic (apolar) substances in the body fat. Most lipophilic compounds also can pass cell membranes fairly easily; hence their excretion into the milk, where they are present in the fat. Consequently, concentrations usually are given per kilogram of fat. Because of the behavior mentioned, several lipophilic contaminants are potentially harmful to the consumer (chronic tox-

icity). Milch animals tend to metabolize and thus consume body fat in order to provide energy and nutrients to produce milk; this may cause an increase in the level of any lipophilic contaminant in the blood, hence in the milk.

2. Materials that are introduced into the udder, notably drugs (like sulfonamides) and antibiotics to treat mastitis, readily contaminate the milk. In most cases their presence in the milk is reduced to negligible quantities within three or four days after they are administered.
3. During milking and transport, milk may become contaminated by substances emitted by the cow (udder, skin, feces), the milker, the air and utensils, pipelines, and so on. Similar contamination may occur during manufacture of milk products and from packing materials. Proper hygiene and the use of suitable materials (stainless steel, glass, and some plastics) will reduce such contamination to negligible amounts. However, milk may take up substances (notably cations) that are adsorbed onto metal and glass surfaces. For example, traces of Cu present in water may deposit on such surfaces and may not be removed fully by normal cleaning but are readily taken up by milk.
4. Substances may be added on purpose; except for the few compounds generally accepted as additives in manufacture (e.g., $CaCl_2$ and $KNO_3$ or $NaNO_3$ in cheese making, NaCl to butter and cheese), this constitutes adulteration. Examples are adding bactericides to slow down microbial deterioration and adding water to boost milk yield.
5. Bacterial action may give rise to compounds that also may enter the milk by contamination, for instance, benzoic acid.

A list of possible contaminants would be almost endless. Widely varying conditions determine what compounds gain entrance into the milk and in what concentrations. Moreover, hitherto unmentioned substances may be detected or receive interest at any moment. Consequently, we will not discuss them all. The list below merely gives a rough classification of milk contaminants that are at the moment chiefly of interest because of their (suspected) toxicity or mutagenicity.

*Pesticides* and chemicals used for comparable purposes (herbicides, fungicides, etc.) probably have aroused the most interest, especially the organochlorine-type insecticides (DDT, aldrin, dieldrin, lindane, and many others). These have been used extensively and, because of their lipophilic nature, tend to accumulate in the fat. Their accumulation factor (concentration in milk fat in $mg \cdot kg^{-1}$ divided by concentration in the feed in $mg \cdot kg^{-1}$) mostly ranges from 0.2–10.0. Though concentrations considerably in excess of the generally accepted maximum limits (0.02–1.25 mg per kilogram of milk fat, according to type of pesticide) have been reported in milk, the levels are now below (and usually much below) the limits in most countries. Organophosphates and carbamates also are used widely as pesticides. Although

they are potentially harmful to the consumer, there is less concern about them because they do not accumulate in the fat and are largely broken down by the cow, predominantly in the digestive tract

*Polychlorinated biphenyls* (PCBS) are potentially harmful for the same reasons as the organochlorine pesticides PCBs have been used widely in industry, causing considerable contamination of the environment in general, and milk has not escaped Their use is now curtailed in many countries

*Plasticizers* from plastics (packing materials, milk pipelines) have been reported in milk, notably some phthalates, likewise, antioxidants from rubber teat cup liners may contaminant milk A proper choice of material suffices to prevent such contamination

*Detergents and disinfectants* may gain entrance readily if milk utensils and manufacturing equipment are not sufficiently rinsed Another source is teat dips, which are disinfectant solutions applied to the teats before or after milking in an attempt to prevent mastitis Detergents are rarely harmful but some disinfectants are Of special concern are iodophores, excessive contamination may increase the iodine in milk to undesirable levels

*Nitrates and nitrites* sometimes are problems Cheese whey may show undesirable levels, because (limited) quantities of nitrate are used widely to prevent late blowing in cheese Nitrate can be reduced to nitrite, which is a suspected precursor of the highly carcinogenic nitrosamines The latter compounds, however, have never been found in milk products in significant quantities

*Antibiotics* have caused, and in some cases still cause, considerable prob lems in some fermentations of milk (notably yogurt) and are not acceptable with regard to the consumer's health

*Drugs* administered to the cow may gain entrance into the milk This also may be the case with synthetic hormones illegally given to boost animal growth, but these are not normally applied to milk-producing cows

*Mycotoxins* in milk usually originate from molds growing on concentrates fed to the cows Particularly suspect are the highly carcinogenic aflatoxins and some of their metabolites Strict control of feed is the best way to minimize the risk

*Heavy metals* that are suspect for toxicological reasons include Pb, Hg, and Cd, but toxic levels have never been found in cow's milk For most heavy metals, the cow is an effective filter

## 8.3. RADIONUCLIDES

We are concerned here with radioactive isotopes of some elements rather than specific chemical compounds Such isotopes are always present in milk but in minute quantities Higher levels are found when considerable radioactive fallout occurs, such as that caused by the use or testing of nuclear

weapons. The consumption of contaminated food (including milk products) may then pose a serious health risk, chiefly possible carcinogenesis. In milk the greatest concern is posed by $^{90}Sr$ and $^{131}I$.

Table 8.2 lists potentially hazardous radionuclides in milk, with some particulars. The physical half-life refers to the period needed to reduce the radioactive emission by an isotope to half of its original level. The biological half-life refers to the time it takes until half of the amount of an element ingested by a person has been excreted. To be sure, the latter is a poorly defined quantity; the element goes to several locations in the body (digestive tract, blood, liver, bone, etc.), and each of these pools has a different half-life. The table refers to the half-life of the most tenaciously held pool, which may concern only a small fraction of the isotope ingested (e.g., 6% for $^{140}Ba$). Even then, the values are uncertain; they depend greatly on physiological conditions and on nutrition. The location of the element in milk indicates which physical fraction of the milk is comparatively safe if serious contamination has occurred.

The cow acts as a filter for radionuclides. Of the daily quantities of I and Cs ingested by the cow, roughly 1% is secreted in every kilogram of milk produced daily. For Ba and Sr the proportion is less. Furthermore, it is not so much the quantity of, say $^{90}Sr$ taken up that is important as the ratio of Sr to Ca. This ratio is reduced by a factor of about 10 by the cow (i.e., from feed to milk).

Strontium accumulates in the bone and stays there for a long time. Consequently, health authorities are particularly apprehensive of $^{90}Sr$ (which also has a long physical half-life) as its uptake causes a prolonged exposure to harmful radiation. Sr is distributed in milk in much the same way as Ca, but because Sr phosphates are less soluble than Ca phosphates, a greater proportion is in the casein micelles. Ba behaves more nearly like Ca.

Iodine accumulates in the thyroid gland, where it attains high concentrations, and radioactive isotopes thus produce intensive radiation. Consequently, $^{131}I$ is considered to be particularly hazardous for comparatively

**Table 8.2. Important Radionuclides That May Occur In Milk**

| Radionuclide | Physical Half-Life | Biological Half-Life[a] | Location in Milk |
|---|---|---|---|
| $^{89}Sr$ | 52 days | 1–50 years | > 80% in casein micelles |
| $^{90}Sr$ | 28 years | | |
| $^{131}I$ | 8 days | 100 days | serum |
| $^{133}I$ | 21 hours | | |
| $^{137}Cs$ | 33 years | ~ 30 days | serum |
| $^{140}Ba$ | 13 days | < 3 years | plasma |

[a]Of the most persistent pool.

short periods after serious fallout. Most I is found in milk serum, a small portion being strongly bound to proteins; about 2% is in the fat globules. Cs chemically behaves like $Na^+$ and $K^+$.

## *Suggested Literature*

Several minor components of milk are discussed by

R Jenness, in *Lactation A Comprehensive Treatise*, B L Larson and V R Smith, eds , vol III, chap 1 (New York Academic Press, 1974)

A H Johnson in *Fundamentals of Dairy Chemistry*, 2nd ed , B H Webb, A H Johnson, and J A Alford, eds , Chap 1 (Westport AVI, 1974)

A review on trace elements in milk is

G K Murthy, *CRC Crit Rev Environ Control* 4 (1974) 1–38

Much information on chemical residues in milk and milk products is in

*International Dairy Federation Bulletin* Document 113, 1979

Environmental contaminants in milk also are discussed by

M Kroger, in *Lactation A Comprehensive Treatise*, B L Larson and V L Smith, eds , vol III, chap 3 (New York Academic Press, 1974)

In the same book, physiological and chemical aspect of radionuclides in milk are treated by

F W Lengemann, R A Wentworth, and C L Comar, chap 4

# 9

# Variability

Cow's milk is remarkably constant in a qualitative sense, but quantitative variation in composition is important. Broadly speaking, the relative variation in the milk yield of a cow is wider than that in composition. The structure of the milk (e.g., the size of the fat globules) and other properties (such as heat stability) vary as well. Since numerous factors affect composition and properties, averages and variability can never be predicted precisely. They depend, of course, on the sample population (e.g., compare samples of individual milkings with lactation averages of cows) and on the type of average (e.g., whether or not weighted with milk yield).

Variation of the gross composition of milk has been studied most extensively. Usually in such studies, *protein* means crude protein (i.e., total N × 6.38) and instead of salts, "ash" content is given. Often lactose content is calculated as the difference between solids-not-fat and crude protein plus ash and is therefore somewhat too high. Sometimes, dry matter content is calculated from fat content and density, and this is not very accurate. The analytical methods employed may thus affect the results. (See further Chapter 20.)

## 9.1. SOURCES AND NATURE OF VARIATION

It is useful to distinguish three kinds of factors leading to variation: genetic (among species, breeds, and individuals), physiological, and environmental (such as feed, climate, and treatment). To be sure, the effects of physiological factors (such as stage of lactation, age, and health) are, in turn, determined by genetics and environment. We will discuss the various sources and some of their effects.

### 9.1.1. Interspecies Variation

By definition all species of mammals produce milk. There are about 4000 extant species of mammals, but only for about 200 is anything known about milk composition. Furthermore, for many of these species only a single sample or a few samples, which may not be representative, have been analyzed. The available data show great interspecies variation in gross composition, in fatty acid patterns, and in the composition of proteins, carbohydrates, and minerals. Dry matter content ranges from 8.0–68%, fat 0.3–53.0%, protein 1.0–24.0%, lactose 0–10.0%, and ash 0.2–2.0%. Fatty acid patterns range from the high short chains and low polyunsaturates of ruminants to the highly unsaturated long-chain compositions of marine mammal milk fat. All milks examined to date contain casein, but its proportion ranges from 25–90% of the total protein. $\beta$-lactoglobulin constitutes 10% of the protein of some ruminant milks and occurs in milks of such other families as horses and carnivores but is absent from primate milks. $\alpha$-lactalbumin is absent or scarcely detectable in milks of certain seal species but is prominent in most other milks. Serum albumin and immunoglobulin contents probably also vary among species, but few quantitative data are available. Not only the total mineral content of milk, as reflected in ash, but also the proportions of the various mineral elements vary greatly among species. The citrate content of milk ranges from virtually none in milks of some rodents to about 0.2% in ruminants.

Presumably milk composition reflects the specific nutritional needs of the young and the natural habitat and nursing habits of the species. There are, indeed, fair correlations between relative growth rate of the young and the protein and calcium phosphate contents of the milk. Animals living in cold arctic and aquatic climates produce milk of high fat content for the obvious reason that fat is needed as a concentrated source of energy and as an insulating material. But desert animals also have high-fat milk, presumably to conserve water. Species that nurse infrequently produce a more concentrated milk than those that nurse frequently. The contents of lactose and $\alpha$-lactalbumin are closely related, undoubtedly because the latter is needed in the synthesis of the former. (See Section 2.3.3.) Some other correlations are not explained so easily. One such is the high correlation in hoofed animals between fat and protein contents of milk. (See Figure 9.1.) Incidentally, intraspecies variation in cows throughout lactation follows reasonably well the interspecies trend shown in Figure 9.1.

The only species raised specifically for milk production, except in laboratories, are hoofed animals. Most important are cow, zebu, buffalo (also called water or swamp buffalo or carabao), goat, and sheep. Western cattle and zebus are considered by some mammalogists to be subspecies of the species *Bos taurus*; crossbreeds between them are used much in an endeavor to combine the high milk production of the former and the endurance of tropical conditions of the latter. Apparently llamas (*Lama glama*) are milked

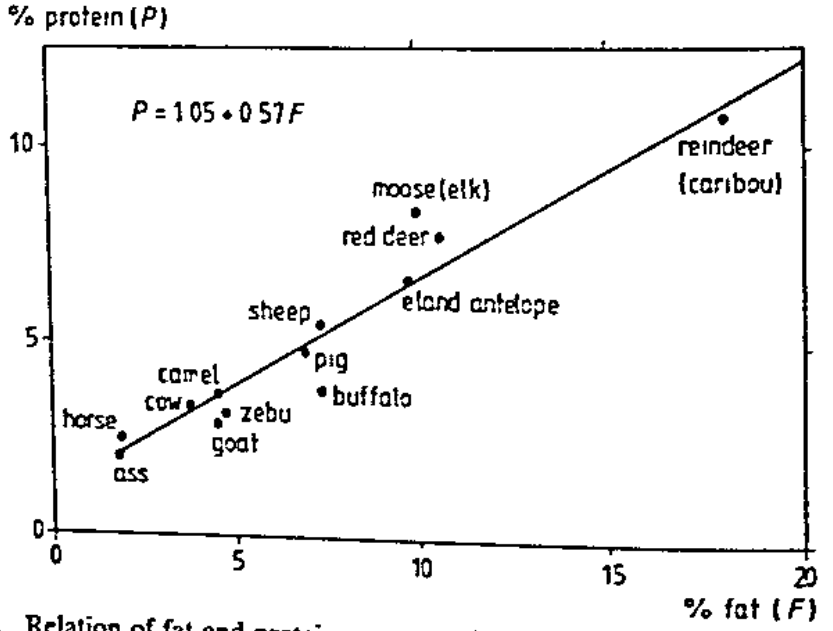



**Figure 9.1.** Relation of fat and protein contents of milks of hoofed mammals. Data of J. R. Luick, R. G. White, A. M. Gau, and R. Jenness, *J. Dairy Sci.* 57 (1974):1325.

occasionally, but we have no reliable data on composition of their milk. Table 9.1 shows that the milks of the species raised for milk production differ markedly in gross composition. The milks of these species differ also in other respects, notably in the characteristics of the milk fat. Goat and sheep milk fats have lower contents of 4:0 acid residues and higher 6:0, 8:0, and 10:0 than that of cow milk; also, they are white because carotene in these species is fully converted into vitamin A. Buffalo's milk also has white fat and comparatively large fat globules. Milks of buffalo, goat, and sheep lack the factors found in cow's milk that cause cold agglutination of fat globules.

Table 9.1 also gives the composition of human milk for comparison; it is discussed more fully in Chapter 19 (Tables 19.1–4) and in the appendix, Table A.25.

### 9.1.2. Variability of Cow's Milk

Most of the figures and tables in this chapter deal with variation in gross composition. The widest variation usually occurs in fat content; variation in protein is less and that in ash still less. (See, for instance, Figure 9.7 and Table 9.3.)

Variation in the fatty acid pattern of milk fat is considerable. (See Sections 2.3.1 and 5.1.) Each individual protein is of constant composition except for genetic variants and such posttranslational modifications as phosyphorylation and glycosylation. (See Section 2.3.2.) The proportions of the several caseins are relatively constant, κ-casein being the most variable. The ratio of casein to whey proteins is fairly constant, but the proportions of individual

**Table 9.1. Approximate Composition (% w/w) of Milk of Humans and some Milch Animals**

| Species | | Water | Fat | Casein | Whey Protein | Lactose | Ash |
|---|---|---|---|---|---|---|---|
| Human | *Homo sapiens* | 87 1 | 4 5 | 0 4 | 0 5 | 7 1 | 0 2 |
| Donkey | *Equus asinus* | 88 3 | 1 4 | 1 0 | 1 0 | 7 4 | 0 5 |
| Horse | *Equus caballus* | 88 8 | 1 9 | 1 3 | 1 2 | 6 2[a] | 0 5 |
| Camel | *Camelus dromedarius*[b] | 86 5 | 4 0 | 2 7 | 0 9 | 5 0 | 0 8 |
| Reindeer | *Rangifer tarandus* | 66 7 | 18 0 | 8 6 | 1 5 | 2 8 | 1 5 |
| Cow | *Bos taurus* | 87 3 | 3 9 | 2 6 | 0 6 | 4 6 | 0 7 |
| Zebu | *Bos indicus* | 86 5 | 4 7 | 2 6 | 0 6 | 4 7 | 0 7 |
| Yak | *Bos grunniens* | 82 7 | 6 5 | -5 8[c]- | | 4 6 | 0 9 |
| Buffalo | *Bubalus bubalis* | 82 8 | 7 4 | 3 2 | 0 6 | 4 8 | 0 8 |
| Goat | *Capra hircus* | 86 7 | 4 5 | 2 6 | 0 6 | 4 3 | 0 8 |
| Sheep | *Ovis aries* | 82 0 | 7 2 | 3 9 | 0 7 | 4 8 | 0 9 |

*Note* After various sources
[a]Calculated by difference
[b]Also *C bactrianus* and cross breeds
[c]Probably 6 38 × total N

whey proteins vary more than those of the caseins Some variation in mineral composition occurs (Figure 9 3 gives examples )

The distribution of components among the physical fractions of milk varies somewhat Fat globule size varies considerably, the volume–surface average $d_{vs}$ ranging from 2 5 to 6 0 $\mu$m, which corresponds to a difference by a factor of 14 in average volume Casein micelles also vary in size, voluminosity (i e , volume per unit mass of dry casein), and composition Within a single milking of one cow, fat globules and casein micelles vary not only in size but also in composition

Variation in composition causes variation in physical properties Standard deviations for individual cow samples throughout a year are roughly 2 kg m $^{3}$ for density, 1 m*M* for titratable acidity, 0 04 units for pH, $10^{-3}$ units for refractive index of the fat, and 5% for viscosity

The following paragraphs present the most important sources of variation

**Breed.** A wide diversity of breeds of cow exists Breeds should not be considered subspecies, they are predominantly the result of selection by people to obtain cattle suitable for the production of milk, meat, or draught power and fit for local conditions, such as climate, feed, terrain, and customs This has led to a wide variability in milk yield and, to a somewhat lesser extent, composition Typical dairy breeds, however, do not show much variation in milk composition Some examples are in Table 9 2, the differ-

**Table 9.2. Approximate Average Composition (% w/w) of the Milk of Some Breeds of Cow**

| Breed | Dry Matter | Fat | Crude Protein | Lactose | Ash |
|---|---|---|---|---|---|
| Friesian[a] | 13.0 | 4.1 | 3.4 | 4.6 | 0.75 |
| Holstein[b] | 12.5 | 3.7 | 3.3 | 4.6 | 0.73 |
| Ayrshire | 12.9 | 4.0 | 3.4 | 4.6 | 0.74 |
| Brown Swiss | 12.9 | 3.9 | 3.5 | 4.6 | 0.75 |
| Jersey | 14.8 | 5.3 | 3.9 | 4.7 | 0.76 |

*Note:* After various sources.
[a]In the Netherlands.
[b]In the U.S.A. and Canada.

ences shown may even be slightly exaggerated, because the data are from various sources, which implies that conditions were not strictly comparable. Selection toward present-day needs and international trade in breeding animals have decreased variation. For example, the typical lowland breeds (like Friesians, Holsteins, Red Danes, and various Red-pied breeds) have increased their milk fat content from less than 3.0% to 3.7–4.1%. Only the milk of the Channel Island breeds (Jersey and Guernsey) still is clearly different, having very high fat and protein contents and large fat globules.

Cows not raised for milking show a wider range in milk composition. Within species variation of buffalo's, goat's, and sheep's milk also is comparatively wide, presumably because selection has been less intense and trade in breeding animals slight.

**Individuals.** Variation in milk composition among individual cows of one breed may be considerable. Examples are given in the next section. (See Table 9.3.) If the animals are from the same herd, the greater part of the variation mostly will be the result of genetic factors.

**Quarters.** Differences in composition in the milk of different quarters of the udder of one cow mostly are negligible, unless a quarter is or has been affected by mastitis.

**Stage of Lactation.** The time elapsed after parturition or calving considerably influences milk composition, presumably because the needs of the calf change with age. To be sure, the time at which the cow becomes pregnant again also affects milk composition and the duration of lactation. It is common to milk cows for ten months and leave them dry for two months, but they can be milked right up to the next calving. Figures 9.2 and 9.3 give examples of lactation curves and show that variation can be considerable.

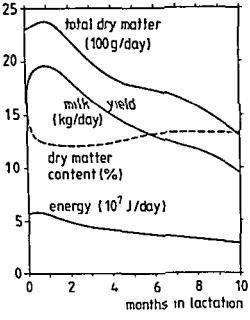



**Figure 9.2.** Approximate average milk yield per cow and total dry matter and energy excreted with the milk during lactation After various sources

Other constituents change as well, some to an even greater extent The pH is on average at first about 6 6, then for a period about 6 7, and rises to about 6 9 at the end, this implies that the $H^+$ activity varies by a factor of 2 In the course of lactation the total ionic strength and the $Ca^{2+}$ activity decrease (especially at first), the proportion of the Ca and the phosphate associated with the casein micelles increases, and the concentration of calcium phosphate in the micelles slightly decreases The quantity of P present in soluble esters decreases from about 250 to about 50 mg $kg^{-1}$ of milk (average ~ 90 mg) The ratio of caseins to whey proteins remains practically constant for many cows (except in colostrum), but the proportions of indi-

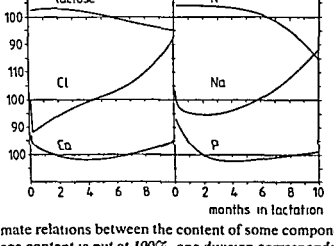



**Figure 9 3** Approximate relations between the content of some components in milk and stage of lactation The average content is put at 100% one division corresponds to 5% After various sources

vidual whey proteins change; for instance, serum albumin increases and immunoglobulins decrease with lactation. The tendency for the fat globules to agglutinate in the cold markedly decreases with lactation. At the very end of lactation milk composition becomes slightly more similar to that of blood serum; the tendency to lipolysis may increase considerably.

The data of Figures 9.2 and 9.3 are approximate averages. The extent to which milk composition changes with lactation varies markedly among cows. (See also Table 9.3.) It is difficult to establish the precise effect of lactation stage; both season and feeding regime may change along with advancing lactation (see below). The effect of lactation stage also depends on the condition of the cow; for instance, if it is well fed at parturition, milk fat content is high at first (as in Figure 9.3), but this is not so if the cow is underfed. If a cow remains barren for a long time after parturition and lactation is thus prolonged, milk composition eventually may become very different, dry matter content being very high (26% has been observed once for a Friesian cow).

**Colostrum.** Figure 9.3 shows that milk composition rapidly changes at the onset of lactation. The first secretion after parturition has a very different composition and is called colostrum or beestings. Figure 9.4 shows how colostrum changes into more or less normal milk in about three days. However, this varies widely among cows, and some show a far less marked transition than given in Figure 9.3, others an even sharper one.

Colostrum is somewhat similar to blood serum and may even contain some red blood cells. It is also high in leukocytes. The protein composition is highly specific; immunoglobulin content in the first colostrum mostly is about 7.0%, as compared to less than 0.1% in midlactation milk. The high serum protein content causes colostrum to gel like eggwhite when heated at, say, 80°C. Cu content is greater than 100 $\mu g \cdot kg^{-1}$, as compared to 20 $\mu g \cdot kg^{-1}$ in midlactation, and Fe content also is high. Enzyme composition is markedly different, colostrum, for instance, containing greater quantities and more species of proteolytic and lipolytic enzymes. The pH can be as low as 6.0.

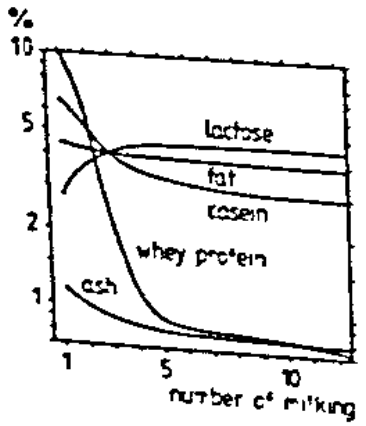



**Figure 9.4.** Example of composition of colostrum and early-lactation milk for successive milkings after parturition. Cows milked twice a day. Percentage by weight, logarithmic scale. After various sources.

**Age of Cow.** This has a very small but consistent effect on milk composition Fat and solids-not-fat contents decrease very slightly with each successive lactation

**Mastitis.** Several species of pathogenic bacteria may cause inflammation of the udder if they have succeeded in entering it, such an inflammation is called mastitis It causes a decrease in milk yield and a change in milk composition, and the number of somatic cells in the milk (especially polymorphonuclear leukocytes) increases A cell count of greater than $5 \times 10^5$ $ml^{-1}$ often is taken as a criterion for mastitis, but this is not precise Other factors affect cell count, notably the individual cow, the stage of lactation, and the age (and perhaps mastitis history) of the cow Examples are given in Figure 9 5 Consequently, an udder quarter without inflammation may give milk with a high cell count (e g , $10^6$ $ml^{-1}$), while another one may have mastitis and a changed milk composition while the cell count is only $2 \times 10^5$ $ml^{-1}$ To be sure, different methods for cell counting may not give matching results, and differences by a factor of 2 are no exception Finally, because of the very wide range in cell counts, the average value obtained for a number of samples greatly depends on the method of averaging For instance, the cell count frequency of quarters in Figure 9 5C gives a numerical average of some $6 \times 10^5$ ml $^{1}$, the average weighted with milk yield (i e , the hypothetical milk composed of all milkings) would have been lower, say $5 \times 10^5$, and the often taken geometric average (obtained from the numerical average of log cell count) is only about $2 \times 10^5$

Figure 9 6 shows typical examples of the relation between milk composition and yield, and cell count If the latter is very high, yield is much reduced and milk composition becomes more similar to that of blood serum This is reflected, for instance, in protein composition, mastitic milk containing as much as 10 times more serum albumin and immunoglobulins as

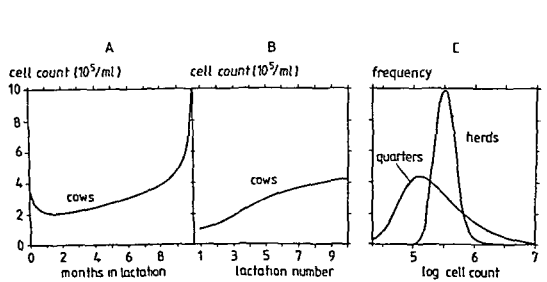



**Figure 9 5** Somatic cell numbers in milk Examples of the cell count as a function of (A) stage of lactation and (B) age of cow frequency (C) of samples of milk from individual quarters and from herds with a certain cell count After various sources

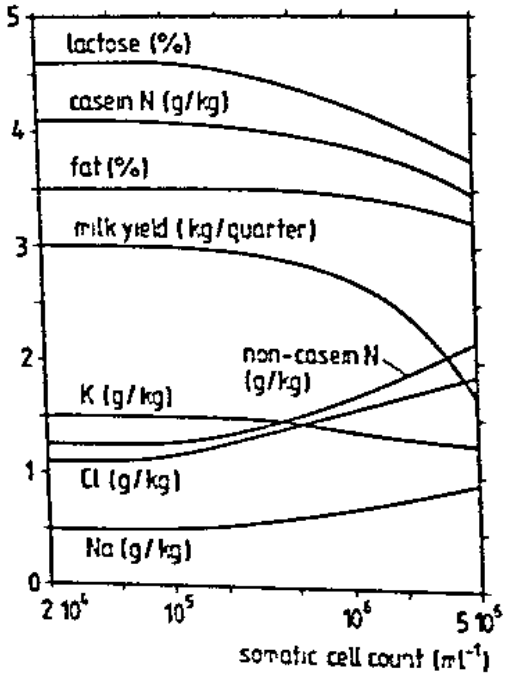



**Figure 9.6.** Examples of the milk yield and the concentration of some components of milk as a function of cell count (logarithmic scale) for samples from individual quarters. After various sources.

normal milk but only about half the amount of $\beta$-lactoglobulin and $\alpha$-lactalbumin. Mastitic milks contain a wider range and higher concentrations of several enzymes, particularly proteinases. Consequently, amounts of proteose–peptone may be 5 times normal, which also implies a change in casein composition (relatively less $\beta$, more $\gamma$); the proportion of $\kappa$-casein may be high and para-$\kappa$-casein may be present. Alkaline phosphatase may show a five-fold and catalase a twenty-fold increase, with much of the latter enzyme originating from the somatic cells. Spontaneous lipolysis seems at first to increase with cell count but to decrease again at very high counts. Hydrogen ion activity decreases, the pH of mastitic milk being about 0.3 units higher. Fat composition is affected, the proportion of short-chain fatty acid residues being somewhat higher in mastitic milk.

The best criterion for mastitis is, of course, the presence or abundance of udder pathogens in the milk. But there is a great demand for quick and easy diagnostic tests. Mostly, cell count is taken as such, but, as discussed, it is somewhat ambiguous. Tests for cellular components (e.g., DNA, catalase) turn out to be less accurate. Possibly a low lactose content or a high electric conductivity (because of the high salt content) may be almost as good an indicator as a high cell count.

**Nutrition.** Because of homeostasis (i.e., the capability of an organism to maintain a constant composition of body fluids and cells, despite gross differences in food), nutrition has a comparatively small effect on most milk constituents. The level of nutrition primarily affects milk yield and has less influence on composition. Section 2.3 gives specific effects of nutrition on udder metabolism and milk composition. Fat content is affected distinctly by the feed, and fat composition is even more so. A high-protein diet causes

nonprotein N content of the milk to increase. Several minor components, such as carotene and its derivative vitamin A, are strongly affected by the content in the feed, and the same is true for I and some other trace elements.

**Climate.** Unless they are extreme, climate or weather conditions have little effect on milk composition. High ambient temperatures (> 30°C) tend to give higher fat, lower N, and lower lactose contents. At temperatures below freezing, fat and N contents both tend to be higher.

**Method of Milking.** During milking the fat content of the milk leaving the udder increases (e.g., from 1–10%), although there are marked differences among cows in this respect. This implies that any milk left in the udder because of incomplete milking will be high in fat content, and so will the milk drawn during the next milking. This largely explains the often encountered day-to-day fluctuations and part of the wide variability in fat content of individual milkings. If the time elapsed after the previous milking is short, milk yield tends to be lower and fat content higher. Hence, evening milk usually has a higher fat content than morning milk, because of a difference in time interval. Other milk constituents are hardly affected by the method of milking.

**Other Factors.** Milk may vary, of course, in composition or properties because of contamination (Section 8.2) and processing, but these aspects are not considered here.

## 9.2. EXTENT OF VARIATION

Natural fluctuations in composition or properties often show a log-normal frequency distribution. Such a distribution presumably results from the existence of many causative variables, each of which may increase or decrease the measured variable (e.g., lactose content) by a certain factor close to 1. (Incidentally, the more commonly applied distribution is the normal or Gaussian one that arises if many variables add or subtract a certain small quantity. This is a less likely mechanism, which also follows from the fact that it allows the existence of negative results, clearly impossible for such variables as lactose content.) For a log-normal distribution a plot of the frequency against the logarithm of the variable shows a symmetrical, Gaussian curve. Examples of distributions are in Figures 9.7 and 9.5C. The distributions of lactose content and cell count of quarters are skewed markedly, the former having a tail to the left, the latter one to the right. The cause is probably mastitis; most samples are from healthy udders (or quarters), but some are not. This is a clear example of a distribution that is composed of two or more different subdistributions, each with its own average.

The extent of variation of a variable $x$ is usually expressed as the standard

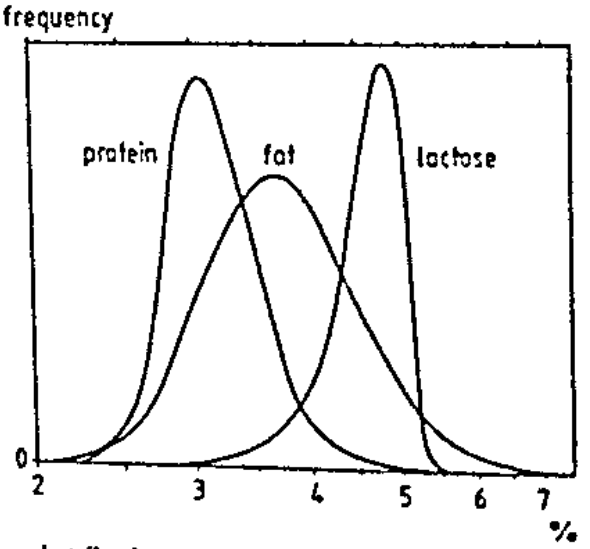



**Figure 9.7.** Frequency distributions of crude protein, fat, and lactose contents (logarithmic scale) of samples of milk from individual cows, taken at random over a large area (of Scotland) throughout a year. After J. F. Tocher, *Variations in the Composition of Milk* (London: H. M. Stationery Office, 1925).

deviation $\sigma(x)$. Because most variables are log-normally distributed, the geometric standard deviation would be more appropriate, but if the variation is not too wide the difference between a log-normal and a normal (Gaussian) distribution is slight. For a widely different variable such as cell count, however, the standard deviation conveys little useful information. The width of a distribution is essentially a relative property (relative to the average value), so the coefficient of variation $c(x) = \sigma(x)/\bar{x}$ is a useful parameter; it is often expressed in percent. Examples are in Table 9.3.

The extent of variation strongly depends on the sample population. A good example is in Figure 9.5C, where quarter and herd samples can be compared. Lots of milk that are composed of a greater number of (hypothetical) sublots of varying origin mostly show a smaller spread, because part of the source of variation is eliminated. The fat content of samples of individual cows may range from 2–9%, but the range in fat content of milk as received at a dairy factory will rarely exceed 1%. Table 9.3 shows that the variation among lactation averages is generally smaller but may in some cases be larger than the variation among samples from one cow. The differences among cows in the variability of components are striking. Consequently, Table 9.3 is meant only to illustrate trends and is by no means valid for all cases; another herd, kept under different conditions, would give different results. Moreover, standard deviations (and correlations) may be calculated in various ways. In Table 9.3, for instance, the differences between evening and morning milk have been eliminated; otherwise, the variations in fat content within cows would have been much wider, whereas those among cows would have been the same.

**Table 9.3. Variation in the Concentration (% w/w) of Some Components**

| | Within Cows | | | Among Cows | |
|---|---|---|---|---|---|
| Component | Average $\sigma(x)$ (% $x$) | Average $c(x)$ (%) | Range of $c(x)$ (%) | $\sigma(v)$ (% $x$) | $c(x)$ (%) |
| Fat | 0 57 | 14 5 | 8–19 | 0 25 | 6 2 |
| Solids-not fat | 0 33 | 3 7 | 3–15 | 0 26 | 2 9 |
| Crude protein | 0 36 | 10 6 | 8–15 | 0 22 | 6 5 |
| Lactose | 0 20 | 4 5 | 3–8 | 0 21 | 4 6 |
| Ash | 0 035 | 4 0 | 3–7 | 0 034 | 3 9 |

*Note* Milk of 10 Friesian cows of one herd throughout a lactation period (10 months), excluding colostrum Variation within cows has been calculated separately for evening and morning milk and then averaged Variation among cows is for yield weighted lactation averages
Source After A J van der Have J R Deen and H Mulder, *Neth Milk Dairy J* 33 (1979) 65 and 82

## 9.3. CORRELATIONS AMONG COMPOSITIONAL PARAMETERS

Many variables in milk composition and properties are correlated Correlation is usually expressed as the product–moment correlation coefficient $r$, which ranges from $-1$ to $+1$, $r = 0$ indicating no correlation Taking as an example the relation between milk protein and fat contents, Table 9 4 gives for the lactation averages of a particular group of cows $r$ (crude protein fat) equals $+0$ 56 Table 9 3 gives for the corresponding standard deviation in crude protein content 0 22%, this is the total standard deviation $\sigma_t$ Part of $\sigma_t$ for protein is thus explained by variation in fat content, but for any fat content there remains a variation in protein content, to be expressed as the standard deviation free from regression $\sigma_f$ We now have the relation

$$r^2 = 1 - \left(\frac{\sigma_f}{\sigma_t}\right)^2 \qquad (9\ 1)$$

It turns out that in this particular case $\sigma_f$ equals 0 18% protein, not so greatly different from $\sigma_t$ In general, $r$ must be quite near 1 or $-1$ for $\sigma_f$ to be small, for a certain value of $\sigma_f$, $r$ will be nearer to 0 if $\sigma_t$ is smaller Equation 9 1 also implies that $r$ depends on the variation width $\sigma_t$, if the sample population shows a smaller spread, the correlation will be less if $\sigma_f$ is the same To be sure, the above relations hold precisely only if the variables are normally distributed

Table 9 4 gives some examples of $r$, and it is seen that many correlations vary widely among cows, moreover, $r$ among cows may be rather different

**Table 9.4. Correlation Coefficient between Concentrations of Some Components or Between Concentration and Yield**

| Correlation Between | Among Cows | Within Cows | | |
|---|---|---|---|---|
| | | Average | Range | |
| Fat and Protein | +0.56 | +0.44 | −0.1 to | +0.8 |
| Fat and Lactose | −0.36 | −0.35 | 0 | −0.8 |
| Total N and Lactose | −0.56 | −0.49 | −0.1 | −0.8 |
| Lactose and Ash | −0.73 | −0.56 | 0 | −0.9 |
| Total N and Casein N | +0.84 | +0.98 | +0.96 | +0.99 |
| Casein N and Ash | +0.45 | +0.67 | +0.3 | +0.9 |
| Yield and Fat | +0.64 | −0.39 | −0.1 | −0.7 |
| Yield and Total N | +0.25 | −0.52 | −0.8 | +0.1 |
| Yield and Lactose | +0.39 | +0.64 | +0.4 | +0.9 |

*Note:* After the same data as in Table 9.3.

from *r* within cows. For example, *r* (total N: casein N) is much higher within cows than between cows, which strongly points to genetic factors being responsible for variation in the protein composition of milk. Another interesting point is that fat and protein contents are positively correlated with milk yield among cows, but mostly negatively within cows. But, again, Table 9.4 is only an example and may not be representative of other sample populations.

Some correlations can be predicted. The constraints on the osmotic pressure of milk cause a strong negative correlation between the concentrations of lactose and dissolved salts. Incidentally, the osmotic pressure is not exactly constant; the coefficient of variation of the freezing-point depression of individual cow samples throughout a year is about 1% (excluding any effects of added water and inaccuracy of determination). Cations and anions in milk must be present in equi-equivalent concentrations. The clear negative correlation between K and Na concentrations (e.g., see Figure 9.3) probably is caused by the same "ion pump" in the basal membrane of the lactating cell transporting K from the blood to the cell and Na from the cell to the blood (which pump is the main means of transport of these ions). The concentrations of casein and undissolved salts are strongly correlated, because the latter are present in the casein micelles. Any component that is part of a group of components, of course, will be correlated with that group, for instance, casein N with total N. Two variables that are both correlated with stage of lactation are generally correlated with each other.

Some correlations are not understood so easily, for instance, that between fat and protein content. The point is of more than academic interest. If the correlation is caused by physiological constraints, any selection by breeders

for the one variable will also change the other Selection for cows with a higher milk fat content has led to an increase in protein content, as would be expected from the correlation between the two (See Table 9 4 ) But it has been shown that the genetic factors determining both variables are not all the same, and selection for the ratio of fat to protein is possible

## 9.4. SOME CONSEQUENCES

Variability in the composition and properties of milk has several conse quences for the dairy industry The industry is particularly interested in seasonal variation, but general trends as to that cannot be given This is because the effect of season depends on climate and feeding practice, which vary widely among geographical regions, and especially because calving pattern may be different If nearly all cows calve within a period of a few weeks, as is customary in a few countries, milk composition and the quantity delivered at the dairy strongly vary with time If, on the other hand, calving dates are evenly distributed over the year gross composition and total milk yield hardly vary with season Consequently, the examples given in Figure 9 8 are by no means generally valid, they have been observed in Western Europe, but even there the trends may be quite different in different regions Regional differences are mainly caused by differences in the predominant breed and in feeding practice Differences between herds are the results of genetic factors (breed of cow, individuals), predominant calving time, feeding practice, and incidence of mastitis

*Yield of products* depends on milk composition, butter yield on fat content, cheese yield primarily on casein content, and yield of skim milk powder on solids not fat Mastitis diminishes the yield of many products, because the ratio of casein N to total N is usually low in mastitic milk, the yield of cheese per percent crude protein also is low

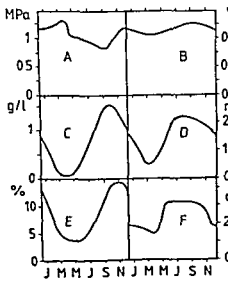



**Figure 9 8** Examples of seasonal variation in properties of milk and milk products (A) Firmness of butter (yield stress) (B) fat content of separated milk (C) quantity of citric acid metabolized by *Leuconostoc cremoris* in milk (D) heat coagulation time of milk at 140°C (E) percentage of herd samples having a fat acidity >1 0 mmol/100 g fat (F) color of milk fat (optical density at 460 nm) After various sources

*Composition of products* likewise depends on milk composition, hence nutritional value and possibly functional properties of the products vary. Variation in fat content usually is counteracted by standardizing the milk. The fat content of skim milk also depends on the efficiency of separation, which in turn depends on fat globule size, which is variable (See Figure 9.8B.)

*Crystallization behavior of milk fat* greatly depends on fat composition, which is especially affected by feed. One consequence is variability in the firmness of butter; an example is shown in Figure 9.8A.

*Heat stability* of milk may vary markedly with season, as illustrated in Figure 9.8D. A main source of the variation is the urea content, but several other factors contribute. The tendency of evaporated milk to age thickening also may show seasonal variation.

*Rennetability* (i.e., the time needed for milk to curdle with rennet) and the properties of the curd formed are rather variable. A main source of variation appears to be the $Ca^{2+}$ activity. Addition of $CaCl_2$ to milk markedly decreases the variability.

*Creaming* of milk, particularly natural creaming in the cold, is very variable. This is caused by differences in agglutinin content, which primarily depends on the cow and on the stage of lactation.

*Emulsion stability* of milk and cream may vary. Sources of variation are probably fat globule size, fat crystallization, and possibly fat globule membrane composition.

Several factors that stimulate or inhibit the *growth and metabolic action of bacteria* (see Section 11.6) are variable. This concerns, for instance, the concentration of agglutinins causing flocculation of some bacteria. The concentration of Mn salts influences the fermentation by some starter organisms (Figure 9.8C) and thereby the preponderance of certain species in mixed starters which, in turn, may affect product properties (e.g., flavor).

*Flavor* of milk may vary considerably, even if flavor defects caused by microbial action are excluded. The balance of salts and lactose is important; the mass ratio 100 [Cl]/[lactose] is often taken as an index, and it varies from 1.2–5.0 for individual cow samples, being high in late lactation and mastitic milk. A high ratio gives a salty taste. Certain feeds impart off-flavors to the milk (Section 18.3.1), and these may depend strongly on season. Lipase activity and, thus, the development of a soapy rancid flavor strongly depends on the cow (variation by a factor of 10 or more) and is more prominent in late lactation. (See also Figure 9.8E.) Proneness of milk to develop flavors caused by fat oxidation (such as "tallowy") also varies among cows; it decreases with advancing lactation.

*Color* of milk and particularly of products like butter and cheese varies considerably because of differences in the carotene content of the fat. This depends on feed (grass containing much and hay containing little carotene) so that season may have a marked effect. (See Figure 9.8F.) It further depends on the ability of the cow to convert carotene into vitamin A, which

strongly varies among breeds (Jerseys giving a highly colored fat) and individuals

Finally, as with any biological matter, variability interferes with the drawing of unequivocal conclusions in such investigations as on the effect of an external variable or treatment on properties of milk or milk products, especially because so many sources of variation exist

## *Suggested Literature*

A survey on milk composition and variability, including species other than the cow, is by

R Jenness in B L Larson and V R Smith eds *Lactation A Comprehensive Treatise* Vol III Chap 1 (New York Academic Press 1974)

A monograph on factors affecting milk composition is

J H Moore and J A F Rook eds *IDF Bulletin* Document 125 1980

Some articles on variability of milk composition are

J C D White and D T Davies *J Dairy Res* 25 (1958) 236

T A Nickerson *J Dairy Sci* 43 (1960) 598

A J van der Have J R Deen and H Mulder *Neth Milk Dairy J* 33 (1979) 65 and 82

The effect of mastitis on milk composition has been reviewed by

B J Kitchen *J Dairy Res* 48 (1981) 167

# 10

# Heating

Milk is nearly always heat treated (i.e., brought to a high temperature for some time and cooled again). Originally, the aim of heating was to make the milk safe for human health by killing pathogenic bacteria, notably *Mycobacterium tuberculosis*. Nowadays, in many countries, the chances of transmitting infectious diseases by raw milk are slight though they should never be neglected. The main purpose of heating milk now is usually preservation, by killing microorganisms, by inactivating enzymes, and by some changes in chemical composition. The latter changes may be partly useful and partly not; many chemical changes occurring at high temperatures are undesirable.

Heat treatment may be applied to impart other desirable properties. In some products, changes in flavor, color, or viscosity caused by heating may be desired. Fermentation of milk by lactic acid bacteria may be much more rapid in heated milk. Heating prior to sterilization may increase the stability of milk to coagulation by later high-heat treatments.

Another purpose of heating is to bring the milk to a temperature at which a process step in manufacturing proceeds as desired, for instance, within a reasonable time. Examples are enzymatic processes like that of chymosin, bacterial fermentation like that in sour milk, evaporation of water from milk, separation of milk into skim milk and cream, and homogenization.

## 10.1 OVERVIEW OF CHANGES

Changes caused by heating may be reversible or irreversible. In this discussion we are interested mainly in irreversible or slowly reversible reactions; such changes scarcely occur at temperatures below 60°C. Nevertheless, rapidly reversible reactions must be taken into account as they may

determine the conditions in the milk at which the irreversible changes take place Examples of reversible reactions are

The mutarotation of lactose (Section 3 2)

Several ionic equilibria (Section 4 5), including pH (Section 11 3)

Conformation of proteins, for instance, the dissociation of the $\beta$-lacto globulin dimer into monomers above 50°C (sometimes designated a pre-denatured state)

Cold agglutination of fat globules (Section 14 3)

State of crystallization of the fat (Section 5 7)

State of association of casein (Section 13 1)

The latter three are of particular importance at, say, 0–40°C

Numerous changes occur on heating, and they are discussed throughout this book Here we give a brief survey, excluding microbiological aspects

1. Gases, including $CO_2$ are removed This happens for any rise in temperature, but it strongly depends on the pressure on the milk during heating The loss of $O_2$ and $N_2$, and to some extent $CO_2$, is reversible But in practice this is often not so, because the milk is held in large or closed containers
2. Some Ca and phosphate are transferred from solution to the colloidal state Though these changes are reversible, the relaxation times are long (See Section 4 5 ) The changes involved somewhat affect size and properties of the casein micelles
3. Lactose undergoes several reactions, causing, for instance, the formation of organic acids mainly formic acid, and of lactulose, these reactions are noticeable only above 100°C (See Section 3 1 )
4. Phosphoric esters, particularly those of casein, are hydrolyzed (See Section 10 3 ) Also some dissolved organic phosphoric esters and even phospholipids may be partly split, at fairly high temperatures (> 100°C)
5. The titratable acidity of the milk increases, and the pH (as measured at room temperature) decreases Examples are given in Figure 10 1, but there are large differences among lots of milk, and the changes may be influenced by other factors as well A main source of acid produced is from lactose (see item 3 above), but several other re actions must contribute or counteract (e g , loss of $CO_2$)
6. The solubility of most of the whey proteins decreases because of heat denaturation (Sections 6 1 and 10 2), and they become largely associated with the casein micelles This starts to become noticeable above 60°C The whey proteins differ in their sensitivity to heating, as is shown in Figure 10 2

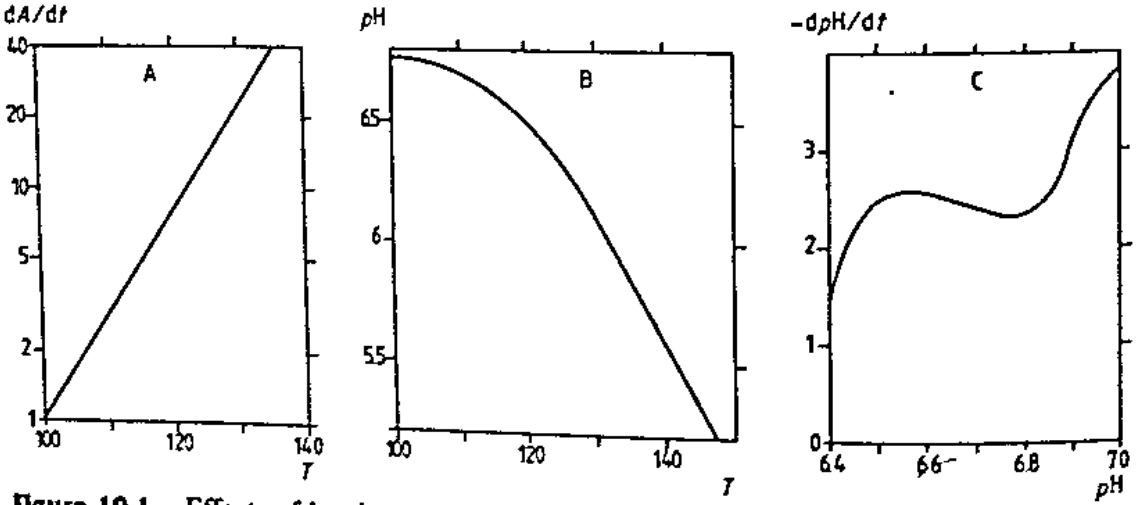



**Figure 10.1.** Effects of heating on the acidity of milk. (A) Acidity produced ($mEq \cdot l^{-1} \cdot h^{-1}$) at various temperatures (°C); (B) pH at room temperature after 30 min heating at various temperatures; (C) rate of change of pH (units per h) at 135°C as a function of initial pH. Examples from various sources.

7. Immunoglobulins become inactive as inhibitors of bacteria and in the cold agglutination of fat globules. The cause is presumably heat denaturation.
8. Enzymes are inactivated. (See Sections 7.4 and 10.2.) This starts at temperatures above 50°C, but it varies greatly among enzymes.
9. Free sulfhydryl groups, and also some hydrogen sulfide, are formed at temperatures above 60°C. (See Section 10.3.)
10. Redox potential drops, probably largely caused by item 9 above. (See Section 11.4.)

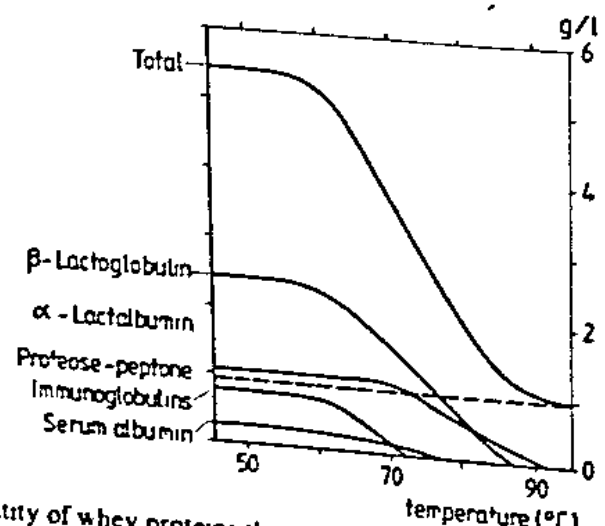



**Figure 10.2.** Quantity of whey proteins that remain dissolved at pH 4.6 after heating milk for 30 min at various temperatures. Mainly after B. L. Larson and G. D. Rolleri, *J. Dairy Sci.* 38 (1955) 351

11. Besides those mentioned under items 4, 6, and 9, numerous changes may occur in proteins, but usually at high temperatures (> 100°C) These are discussed in Section 10 3
12. Maillard reactions occur at any temperature but proceed much more rapidly at higher ones (See Section 10 4 )
13. Casein micelles may start to aggregate above 110°C Numerous reactions are involved, and this is discussed in Section 13 5
14. Some changes occur in the fat globule membrane (e g , its Cu content increases—Section 5 3 4) The proteins of the membrane may undergo changes as indicated under items 6, 8, 9, 11, and 12, the lipids those under items 4 and 15
15. Triglycerides may undergo some interesterification according to the presence of di and monoglycerides, but only at very high temperatures (e g , > 120°C) Some polar lipids undergo changes, too, but little is known about this
16. Lactones and methyl ketones are formed from the fat (See Section 5 2 ) It happens over a wide range of temperatures, and degradation of said substances occurs as well, but in general, higher temperatures give more lactones (hence a more pronounced flavor) Several conditions affect these reactions
17. Some vitamins are partly degraded at high temperatures (See Section 19 6 )

Heat treatments may thus have numerous effects on properties of the milk, besides the change in keeping quality originating from lowering bacterial counts and inactivating enzymes Some of the more important ones are as follows

a. The color at first becomes a little whiter (the cause is uncertain), then browner (from item 12 above)
b The flavor changes, at first a "cooked" flavor develops, mainly from item 9 above, then a much more intense, sterilized milk flavor, from items 12 and 16, and numerous other reactions (See further Section 18 3 )
c. The texture may change Viscosity may increase slightly from item 6 above and much more from item 13 at high temperatures, particularly in concentrated milk Heat treatment by steam injection may cause a "mealy mouthfeel "
d Nutritional value may decrease somewhat, caused by items 11, 12, and 17 above (See further Chapter 19 )
e The suitability of milk as a substrate for bacteria (Section 11 6) may be much improved, though this differs widely according to heating intensity and species or strain of bacterium

f. The tendency for gelation to occur during storage (Section 13.4) and for heat coagulation during sterilizing (Section 13.5) may be diminished.

g. Heat treatment may have several effects on the clotting of milk by rennet or acid. (See Section 13.3.5.)

h. Creaming tendency of the milk may be much less, caused by item 7. (See Section 14.5.)

i. The stability of the fat globules to coalescence may be somewhat diminished. (See Section 14.2.)

j. The rate of autoxidation of the fat in milk products may be much diminished. (See Section 5.6.) This is mainly because of the changes mentioned under items 9, 10, and 14 above.

k. The composition of the surface layers of the fat globules formed during homogenization or recombination is much affected by the intensity of heating before homogenization, mainly by means of item 6. (See Section 14.4.2.) This affects some properties of products, like the heat stability of cream.

l. The tendency of cream to form fat globule clusters during homogenization (Section 14.4.3) can be enhanced by prior heat treatment, probably by means of item 6.

The effects of heating may depend on pH, $O_2$ content, dry matter content (as affected by evaporation), fat content (as affected by centrifugation), and size and membrane of the fat globules (as affected by homogenization). The main variable is, of course, heating intensity (i.e., temperature and duration of heating). Many combinations of time and temperature may thus be of the same intensity (i.e., cause the same extent of reaction), but the combinations are usually different for different reactions. This is further discussed in Section 10.2. In industrial practice, temperatures up to 150°C and duration (holding times) ranging from 1 s to 1 h are applied, though there is a strong tendency to use flowthrough processes, and this implies fairly short durations (say < 1 min). The following are customary processes.

**Thermization.** This is a heat treatment of lower intensity than low pasteurization (e.g., a few seconds at 65°C). The main purpose is to kill most psychrotrophic bacteria, as some of these produce very heat-resistant lipases and proteinases that may eventually cause deterioration of milk products (even if sterilized) if the psychrotrophic count should become high before processing. Thermization causes almost no other irreversible changes.

**Low Pasteurization.** In most countries this is defined legally as a heat treatment of such intensity that the alkaline phosphatase of milk (EC 3.1.3.1) is inactivated. It may be realized by heating for 30 min at 63°C or for 15 s at 72°C. Almost all pathogenic organisms that can be present in milk are killed. Most but not all vegetative microorganisms are killed. There is very

little other permanent change except for inactivation of some enzymes. Flavor is hardly influenced, whey proteins remain unchanged, and cold agglutination (hence rapid creaming) and bacteriostatic properties remain intact. But 15 s at 72°C is insufficient to suppress lipase action fully in homogenized milk. Consequently, a more intense heat treatment is often applied (e.g., 20 s at 75°C). This causes, for instance, a perceptible change in flavor and impaired agglutinative power (of bacteria and of fat globules in the cold).

**High Pasteurization.** In many countries this implies a heat treatment such that lactoperoxidase (EC 1.11.1.7) is inactivated, for which 20 s at 85°C suffices; but higher temperatures, up to 100°C, are sometimes applied. Almost all vegetative microorganisms are killed but not bacterial spores. Most enzymes are inactivated but not milk proteinase and some bacterial lipases and proteinases. Bacteriostatic systems are largely inactivated, and high pasteurization is applied commonly in the manufacture of fermented milk. Part of the whey proteins become less soluble. A cooked flavor arises. There are no significant changes in nutritive value.

**Sterilization.** This is meant to kill all microorganisms, including spores. To that end 30 min at 110°C (i.e., autoclaving), 30 s at 130°C, or 1 s at 145°C usually suffices, though it naturally depends on type and number of spores present. Flowthrough (hence short duration) sterilization is commonly called UHT (ultra-high-temperature) treatment. If the duration is only a few seconds it does not suffice to inactivate all enzymes, even at 150°C. Sterilization, particularly batch sterilization (e.g., 20 min at 115°C), causes severe chemical changes. Color and flavor are appreciably affected, and nutritive value is somewhat diminished.

**"Preheating."** This may mean almost anything from very mild to quite intense heating. Moreover, milk often is heated during other processes, as in evaporation.

It should be realized that there may be a considerable spread in duration and temperature of heating among particles of milk. Also, the times needed for warming and cooling may not be negligible, particularly when the holding time is very short. Figure 10.3 gives some examples. Along with the heating the milk is usually agitated, and this may affect coalescence or disruption of fat globules and the extent of heat coagulation. During boiling and steam injection, agitation is usually quite vigorous.

## 10.2 KINETICS

The reactions that occur in milk are accelerated to different extents by increasing the temperature. Many chemical reactions are accelerated two- or three-fold by raising the temperature 10°C. On the other hand, the rate of denaturation of a globular protein may be increased one hundred-fold or

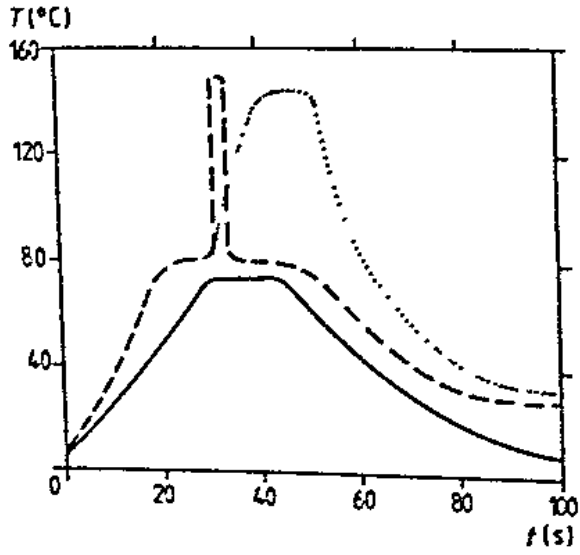



**Figure 10.3.** Examples of the course of the temperature ($T$) of the milk as a function of time ($t$) during various heat treatments: —— low pasteurization; — direct UHT heating (i e., steam injection followed by evaporative cooling); · · · indirect UHT heating.

more by an increase of 10°C. Many of the important reactions induced in milk by heating involve denaturation of proteins, so this aspect is stressed and introduced first. Several reactions not involving protein denaturation are included for comparison. (See Table 10.2.)

As discussed in Section 6.1, denaturation is a change in the secondary and tertiary structure of a protein, essentially (partial) unfolding of the peptide chain. The unfolding as such is reversible, but it exposes many reactive side groups that now may indeed react, particularly at high temperature. This often leads to irreversible changes in the protein. Consequently, globular proteins may become insoluble, enzymes may lose their activity, and microorganisms may be killed (presumably because some crucial enzyme is inactivated). The rate at which these changes occur often is determined by the rate of denaturation.

Denaturation is a first-order reaction: Every protein molecule reacts independently. We thus have

$$-\mathrm{d}c/\mathrm{d}t = k^* c \tag{10.1}$$

where $c$ is the concentration, $t$ is time, and $k^*$ the rate constant (in $s^{-1}$). (Strictly, the thermodynamic activity rather than the concentration should be used.) Integration of Equation 10.1 yields

$$\ln(c_0/c) = k^* t \tag{10.2}$$

where $c_0 = c$ for $t = 0$. Thus, $\log c$ will linearly decrease with $t$; if $t_1$ seconds are needed to cause 90% denaturation, then 99% is reached in $2t_1$ seconds, and so on.

Many conditions affect $k^*$, notably temperature. According to Eyring's absolute rate theory, we have

$$k^* = \frac{kT}{h} \exp\left(-\frac{\Delta G^*}{RT}\right) \tag{10.3}$$

where $k$ is the Boltzmann constant ($1.38 \times 10^{-23}$ J K$^{-1}$), $h$ is Planck's constant ($0.66 \times 10^{-33}$ J s), $T$ is absolute temperature, and $R$ is the gas constant (8.31 J K$^{-1}$ mol$^{-1}$) $\Delta G^*$ is the Gibbs free energy of activation for the reaction

Remembering that

$$\Delta G = \Delta H - T\Delta S \tag{10.4}$$

where $H$ is enthalpy and $S$ is entropy, combination yields

$$k^* = \frac{kT}{h} \exp\left(-\frac{\Delta H^*}{RT}\right) \exp\left(\frac{\Delta S^*}{R}\right) \tag{10.5}$$

Because changes in volume or pressure are usually negligible, we may put $\Delta H^*$ equal to the activation energy. Often it is tacitly assumed that $\Delta H^*$ and $\Delta S^*$ are independent of temperature. Consequently, a plot of $\log(k^*/T)$ versus $1/T$ should give a straight line, the slope of which gives the activation energy.

For many reactions the activation entropy $\Delta S^*$ is small, but this is not true for protein denaturation. The reason is the large increase in conformational entropy occurring on unfolding of the peptide chain. In first approximation, $\Delta S^*$ is proportional to molecular weight. The same holds true for $\Delta H^*$, which is caused largely by the breaking of numerous fairly weak bonds (hydrophobic interactions, H bonds, internal salt bridges). Examples are given in Table 10.1. The large activation energy causes a denaturation reaction to depend very closely on temperature, but it also would cause the reaction to be immeasurably slow if it were not largely compensated for by the positive activation entropy. It turns out that most proteins show fairly rapid heat denaturation somewhere between 60–90°C.

The rate constant $k^*$ may depend on several other factors, such as pH, ionic strength, water activity, and redox potential. Usually, both $\Delta H^*$ and $\Delta S^*$ vary, this implies that the temperature dependence of $k^*$ also changes with conditions. Heat denaturation is usually slower if the pH is nearer the isoelectric pH of the protein. Many enzymes are protected from heat den-

**Table 10.1. Activation Enthalpy (≈ energy) ($\Delta H^*$), Entropy ($\Delta S^*$), and Free Energy ($\Delta G^*$) in the Heat Denaturation of Some Proteins**

| Protein | Molecular Weight | $\Delta H^*$ kJ mol$^{-1}$ | $\Delta S^*$ J mol$^{-1}$ K$^{-1}$ | $\Delta G^*$ kJ mol$^{-1}$ |
|---|---|---|---|---|
| Insulin | 5 800 | 150 | 100 | 113 |
| Hemoglobin | 17,000 | 316 | 640 | 103 |
| Chymosin | 31,000 | 375 | 870 | 94 |
| Alkaline Phosphatase | 40 000 | 450 | 1,000 | 108 |
| Lactoperoxidase | 82 000 | 775 | 1 950 | 106 |

*Note* Approximate data after various sources

aturation by the presence of substrate. Other protecting or accelerating substances may be present. Low water activity ($a_w$) usually strongly reduces denaturation rate. For example, the heat inactivation of alkaline phosphatase in skim milk at 80°C roughly shows $\log k^* = 5a_w - 4$, where $k^*$ is in $s^{-1}$; this implies an almost 100,000-fold reduction in rate when going from milk to milk powder.

Often, reaction kinetics is more complicated than stated above:

1. The protein may be a mixture of different molecules, each with its own rate and temperature dependence of heat denaturation. We may think of genetic variants of a certain protein, or of the isozymes of an enzyme, but also of a mixture like the whey proteins of milk. Bacteria of the same species often show a marked range in heat resistance. In all of these cases, plots of $\log c$ versus time are curved (i.e., the slope, hence $k^*$, diminishes with time). Moreover, plots of $\log k^*$ versus $T^{-1}$ are curved (i.e., the apparent average activation energy increases with temperature).
2. Activation energy and entropy may not be independent of temperature. An obvious reason is that other conditions, like pH or the concentration of protective substances, depend on temperature (and perhaps on duration of heating).
3. The change occurring may involve some consecutive reactions, each with its own characteristics; under different conditions a different reaction may be the rate-determining one. Denaturation often occurs in a few distinct steps.

Heat inactivation of cold agglutination ($\Delta H^* \approx 400$ kJ·mol$^{-1}$) and of most (though not all) enzymes in milk are in good agreement with the theory for heat denaturation. But not all heat-induced changes in milk proteins follow the rules. As an example, the reaction rates for the heat denaturation (as determined from loss of solubility at pH 4.6) of the principal whey proteins, $\beta$-lactoglobulin and $\alpha$-lactalbumin, are given in Figure 10.4. The situation is indeed fairly complicated. For $\alpha$-lactalbumin above 90°C, we derive $\Delta H^* \approx 30$ kJ·mol$^{-1}$ ($Q_{10} \approx 1.3$) and $\Delta S^* < 0$; it is thus not typical for heat denaturation. For $\beta$-lactoglobulin below 90°C, we have $\Delta H^* \approx 280$ kJ·mol$^{-1}$ and $\Delta S^* \approx 510$ J·K$^{-1}$·mol$^{-1}$, which is fairly typical for heat denaturation; but the reaction is second order with respect to time (i.e., $\frac{1}{c}$ is linear with $t$), though first order with respect to concentration. The reaction rate also depends on pH.

The heat denaturation of $\beta$-lactoglobulin shows another peculiarity in that a second denaturation occurs at temperatures near 140°C; calorimetric analysis shows a sharp endothermic transition near 140°C (particularly at somewhat elevated pH), which must be ascribed to a further unfolding of the peptide chain.

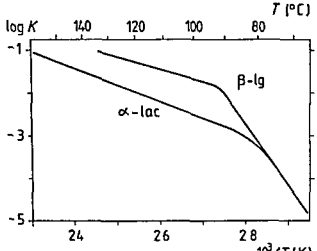



**Figure 10.4.** Reaction constant ($k^*$ for denaturation of $\beta$-lactoglobulin ($k^*$ in $m^3 \cdot kg^{-1} \cdot s^{-1}$) and $\alpha$-lactalbumin ($k^*$ in $s^{-1}$) in milk at various temperatures. After R. L. J. Lyster, *J. Dairy Res.* 37 (1970):233.

In practice we often do not determine $k^*$, but rather the time $t'$ needed to make a protein insoluble or an enzyme inactive. The $t'$ naturally depends on the sensitivity of the assay method employed; it is often on the order of 2% of the original concentration (hence $k^*$ would be something like $4/t'$), but this may vary widely. We usually plot $\log t'$ against $T$ (in °C), and often a fairly straight line is obtained if the temperature range is not too wide. This relation (which also holds if the reaction is not first order) is, however, not precise; equation 10.5 predicts a slightly curved plot, but because of several small uncertainties a more rigorous way of plotting usually does not give a better fit between theory and results. On the other hand, linear extrapolation of a plot of $\log t'$ versus $T$ usually is not justified.

Examples of the temperature dependence of some reactions that may occur in milk on heating are given in Figure 10.5. Other examples of enzyme inactivation are in Figure 7.2. Most changes caused by denaturation are indeed strongly temperature dependent, but there are notable exceptions. Heat inactivation of milk proteinase, some bacterial lipases and proteinases, and, to a lesser extent, milk lipase depend much less closely on temperature (if in the presence of substrate). Consequently, it is questionable whether unfolding of the peptide chain is the cause of inactivation. The plots for whey proteins are markedly curved, which is partly because of the peculiarities shown in Figure 10.4 and partly the fact that a protein mixture is involved. (See Figure 10.2.) The development of a just perceptible cooked flavor in milk is given approximately by the curve for 10% insolubilization.

Figure 10.5 also gives some changes not depending on heat denaturation, like browning and heat coagulation. These changes depend much less on temperature and are more like "ordinary" chemical reactions. Some examples important for milk are in Table 10.2. It should be realized, however, that chemical changes in milk often involve many separate reactions, each with its own $\Delta H^*$ and $\Delta S^*$, which may depend in different ways on environmental conditions; and it may therefore depend on conditions which reaction is rate determining. Consequently, an activation energy calculated from the temperature dependence of reaction rate is only an apparent, average value.

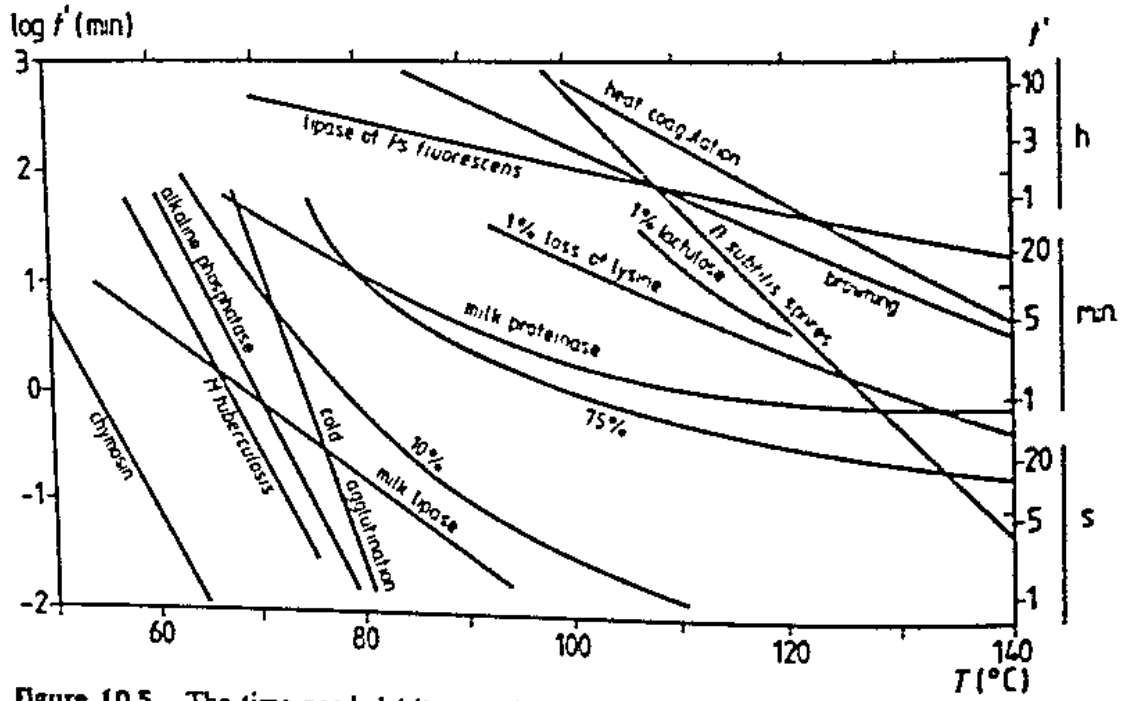



**Figure 10.5.** The time needed ($t'$) at various temperatures ($T$) to inactivate some enzymes and cold agglutination; to kill some bacteria and spores (*B. subtilus* reduced to $10^{-7}$); to cause a certain degree of browning; to convert 1% of lactose to lactulose; to cause heat coagulation; to reduce available lysine by 1%; to make 10% and 75% of the whey proteins insoluble at pH 4.6. Approximate results after various sources.

## Table 10.2. Approximate Examples of the Temperature Dependence of Some Reactions

| Reaction | Activation Energy[a] ($kJ \cdot mol^{-1}$) | $Q_{10}$ at 100°C |
|---|---|---|
| Many chemical reactions | 80–130 | 2.0–3.0 |
| Many enzyme-catalyzed reactions | 40–60 | 1.4–1.7 |
| Hydrolysis, for example | 60 | 1.7 |
| Autoxidation of lipids | 40–100 | 1.4–2.5 |
| Maillard reactions (browning) | 100–180 | 2.4–5.0 |
| Dephosphorylation of caseinate | 110–120 | 2.6–2.8 |
| Heat coagulation of milk | 150 | 3.7 |
| Degradation of ascorbic acid | 60–120 | 1.7–2.8 |
| Heat denaturation of protein | 200–600 | 6.0–175.0 |
| Typical enzyme inactivation | 450 | 50.0 |
| Inactivation of milk proteinase | 75 | 1.9 |
| Killing vegetative bacteria | 200–600 | 6.0–175.0 |
| Killing of spores | 250–330 | 9.0–17.0 |

*Note:* After various sources.
[a]Often an apparent or average value.

The chemical changes often will not follow first-order kinetics With reactions that proceed only to a small fraction of completion, the difference between first- and higher-order kinetics with respect to time may be small But the rate of bimolecular reactions always will increase more steeply with concentration of the milk This is, for instance, prominent for browning (See Section 10 4 )

The great difference in temperature dependence provides the opportunity to kill many bacteria or spores, or to inactivate many enzymes, without inducing severe chemical changes The way to do this is to heat at a high temperature for a very short time The UHT process was invented to sterilize milk without causing appreciable browning or cooked flavor The unexpected high heat resistance of some lipases and proteinases often puts a limit on this practice

Finally, we briefly explain some often used parameters The temperature dependence of a reaction often is given as $Q_{10}$, defined as the factor by which $dc/dt$ is increased if the temperature is raised by 10°C Consequently,

$$Q_{10} = \frac{k^*_{T+5}}{k^*_{T-5}} \approx 1\ 03 \exp\left(10\,\frac{\Delta H^*}{RT^2}\right) \tag{10 6}$$

$Q_{10}$ depends on $T$, the more so when $\Delta H^*$ is large For the killing of micro organisms, we often use decimal reduction time $D$, defined as the time needed to reduce $c$ to 0 1 $c_0$ For a first-order reaction, $D = 2\ 3/k^*$, and for a second-order reaction $D = 9/c_0k^*$ The sterilizing effect is defined as the number of decimal reductions obtained by a certain heat treatment, it equals log $(c_0/c)$ The $Z$ value is the temperature rise (in K) needed to increase the reaction rate by a factor of 10 Consequently,

$$Z = \frac{10}{\log Q_{10}} \approx 2\ 3\,\frac{RT^2}{\Delta H^*} \tag{10 7}$$

and it thus depends on temperature

## 10.3. REACTIONS OF PROTEINS

Heating of proteins in solution promotes certain reactions involving serine phosphate, thiol, disulfide, lysine, and amide side chains, and may also cause cleavage of some peptide bonds The side chains in caseins are readily available for heat-induced reactions, and those of the globular whey proteins are made available by the uncoiling and unfolding resulting from denaturation (See Section 10 2) The peptide bonds of the polypeptide chain are primarily in *trans* configuration, but some isomerization to the less stable *cis* structure is possible if the peptide chain is unfolded Proline residues are an exception in that the *cis* and *trans* configurations have comparable stability, and isomerization is thus much more likely to persist Such isomerization possibly may influence rates of reactions that occur in the protein

and prevent return to the native (undenatured) state after cooling again. Data on the various reactions are somewhat fragmentary; it is not possible as yet to compare them all on the basis of fundamental parameters such as energy of activation. The following descriptions present results that have been observed in experiments in which milk or milk proteins were heated under specific conditions of time and temperature.

Heating milk at 120°C for 30 min (e.g., to sterilize it) may increase proteose–peptone nitrogen (PPN) from 4.7% to 6.0% of total nitrogen. The proteose–peptone fraction of unsterilized milk consists of proteolytic fragments of β-casein and fat globule membrane protein (Section 6.4.7), which are not precipitated at pH 4.6 from a sample of milk that has been boiled. They are precipitated from a pH 4.6 filtrate by adding 120 g $Na_2SO_4$ per liter. The additional proteose–peptone components produced by sterilizing milk most probably arise from hydrolytic cleavage of peptide bonds, but neither the specific bonds cleaved nor the identity of the peptides has been elucidated.

Heating milk at 120°C for 30 min likewise increases its nonprotein nitrogen (NPN) from about 5.5% to 7.5% of total N. This fraction consists of N-containing compounds soluble in 12% trichloroacetic acid. The increase in this category by heating is likely the result of release of ammonia by deamidation of glutamine and asparagine groups of the proteins (reactions 1 and 2, Table 10.3). There is no direct proof that this is indeed the case, but heating caseinate solutions (pH 6.9) at 135°C for 30 min releases an amount of NPN nearly equivalent to the amide N of the protein. Heat treatments used for pasteurization and probably UHT do not cause detectable increases in PPN or NPN.

Heat treatment cleaves phosphate from phosphoserine groups of the caseins; temperatures of 100–140°C for times up to 1 h are effective in producing this effect in skim milk or in caseinate solutions. The rate of heat-induced phosphate cleavage of casein increases somewhat as pH is raised from 6.0 to 7.0. The energy of activation for the reaction at the pH of milk is about 117 $kJ \cdot mol^{-1}$ for sodium caseinate and about 109 $kJ \cdot mol^{-1}$ for calcium caseinate or casein in milk. Treatment at 135°C for 1 h cleaves about 80% of the casein-bound phosphate of skim milk and 90–95% of that in sodium caseinate solutions. Two possible mechanisms of cleavage are hydrolysis, yielding serine residues (reaction 3), and β-elimination, producing dehydroalanine (reaction 4). β-elimination certainly occurs in alkaline solution (e.g., in casein in 0.1 *N* NaOH at 30°C). Hydrolysis would be expected to predominate at low pH. Whether one or both reactions occur upon heating casein solutions or milk at pH 6.6 could be determined easily by analyzing for serine and dehydroalanine after heating, but apparently such analyses have not been made.

There is some evidence that N-acetylneuraminic acid residues and possibly other glycosidic groups are cleaved from κ-casein at high temperatures. κ-casein heated in solution at sterilization temperatures slowly loses its abil-

**Table 10.3. Possible Reactions of Side-Chain Residues of Proteins at High Temperature**

| | | | |
|---|---|---|---|
| 1 | $\vdash CH_2—CONH_2 + H_2O$ | $\longrightarrow$ | $\vdash CH_2—COOH + NH_3$ |
| | Asparagine | | Aspartic acid |
| 2 | $\vdash (CH_2)_2—CONH_2 + H_2O$ | $\longrightarrow$ | $\vdash (CH_2)_2—COOH + NH_3$ |
| | Glutamine | | Glutamic acid |
| 3 | $\vdash CH_2—O—PO_3^{2-} + H_2O$ | $\longrightarrow$ | $\vdash CH_2—OH + HPO_4^{2-}$ |
| | Phosphoserine | | Serine |
| 4 | $\vdash CH_2—O—PO_3^{2-}$ | $\longrightarrow$ | $\models CH_2 + HPO_4^{2-}$ |
| | Phosphoserine | | Dehydroalanine |
| 5 | $\vdash CH_2—SH + OH$ | $\longrightarrow$ | $\vdash CH_2—S + H_2O$ |
| | Cysteine | | |
| 6 | $\vdash CH_2—S$ <br> $\quad\quad S—CH_2\dashv$ <br> $\vdash CH_2—S$ | $\longrightarrow$ | $\vdash CH—S$ <br> $\quad\quad S—CH_2\dashv$ <br> $\vdash CH_2—S$ |
| 7 | $\vdash CH_2—S + S—CH_2\dashv$ | $\xrightarrow{e^-}$ | $\vdash CH_2—S—S—CH_2\dashv$ |
| | Cysteine | | Cystine |
| 8 | $\vdash CH_2—S^-$ | $\longrightarrow$ | $\models CH_2 + HS^-$ |
| | Cysteine | | Dehydroalanine |
| 9 | $\models CH_2 + HS—CH_2\dashv$ | $\longrightarrow$ | $\vdash CH—S—CH_2\dashv$ |
| | | | Lanthionine |
| 10 | $\vdash (CH_2)_4—NH_3^+ + H_2C\models$ <br> $+ OH^-$ | $\longrightarrow$ | $\vdash (CH_2)_4—NH—CH_2\dashv + H_2O$ |
| | Lysine | | Lysinoalanine |
| 11 | $\vdash (CH_2)_4—NH_3^+ + O_2C—CH_2\dashv$ | $\longrightarrow$ | $\vdash (CH_2)_4—NH—CO—CH_2\dashv$ <br> $+ H_2O$ |
| | Lysine Aspartic acid | | $\epsilon$ N ($\beta$ aspartyl)lysine |
| 12 | $\vdash (CH_2)_4—NH_3^+ + O_2C—(CH_2)_2\dashv$ | $\longrightarrow$ | $\vdash (CH_2)_4—NH—CO—(CH_2)_2\dashv$ <br> $+ H_2O$ |
| | Lysine Glutamic acid | | $\epsilon$ N ($\gamma$-glutamyl)lysine |

ity to stabilize the other caseins against $Ca^{2+}$ ions In a broad, sense, $\kappa$-casein appears to be more heat sensitive than $\beta$-casein

The thiol (or sulfhydryl) groups of cysteine residues in proteins are weakly acidic ($pK \approx 9.5$ at 25°C) and probably ionize to greater extents at the pH of milk as the temperature is raised Thiols can participate in several reactions, as indicated in Table 10 3 Uncombined thiols are present in native $\beta$-lactoglobulin, serum albumin and fat globule membrane proteins and disulfides in these, and also in $\alpha$ lactalbumin and immunoglobulins $\kappa$ casein has two Cys residues per mole probably in disulfide linkage, the same holds true for $\alpha_s$-casein In $\beta$-lactoglobulin there is some evidence of an equilibrium between disulfides Cys 106–Cys 119, Cys 106–Cys 121, and thiols Cys 119 and Cys 121 Upon heating at temperatures above 60°C thiols are ex-

posed by unfolding of the protein and become available for reaction with various reagents. Such reactive thiols have sometimes been called free sulfhydryl groups. Their liberation has been studied much as an index of the denaturation process.

If the heating is done in the presence of air, some of the liberated thiols are oxidized to disulfides. Loss of thiol and gain of disulfide groups increase progressively as temperatures of 20-min heat treatments are increased from 60°C to 125°C. As pointed out in Section 7.3.1, milk contains a sulfhydryl oxidase capable of oxidizing thiols to disulfides; the possibility has not been investigated that this enzyme catalyzes formation of disulfide in milk during heating before it is itself denatured. Cysteine residues can be oxidized further than disulfides through various stages to cysteic acid ($-SO_3H$) groups. It is not known whether oxidation beyond the disulfide stage occurs on heating milk proteins. Disulfide formation can be either intra- or intermolecular, the latter constituting a polymerization. Polymerization also can result from disulfide interchange (reaction 6). This has been demonstrated in β-lactoglobulin heated above 70°C and by copolymerization of binary and tertiary systems containing β-lactoglobulin, α-lactalbumin, and κ-casein. Heat-denatured β-lactoglobulin readily reacts with κ-casein, also at room temperature.

When milk is heated above 70°C, β-lactoglobulin reacts with κ- and $\alpha_s$-caseins, and thus becomes associated with the casein micelles or partly with nonmicellar casein if the pH during heating is above 6.6 (as measured at room temperature); at higher pH the association becomes progressively less, and it is virtually absent above pH 7.0. α-lactalbumin, serum albumin, and immunoglobulins also become associated with the casein. These reactions considerably affect the properties of the casein micelles. (See Chapter 13.) If the casein of heated milk is precipitated, for instance, at the isoelectric pH, the heat denaturable whey proteins are included in the precipitate. This observation has been applied in various tests to estimate the extent of heat treatment of milk. When whey is heated, the denatured whey proteins aggregate to some extent, but not much above pH 6.6. At lower pH, their stability at first decreases to reach a minimum near pH 4.5. (See also Section 17.7.)

Cleavage of cysteine thiols by β-elimination to form dehydroalanine residues occurs upon heating (reaction 8). Heating a β-lactoglobulin solution at pH 6.9 for 80 min at 97°C, for example, produces about 0.15 mol dehydroalanine per mole of protein, equivalent to about 15% of the Cys residues; cleavage of half of the Cys may occur upon heating at pH 9.8 for the same time and temperature. There is some evidence that the β-elimination occurs only after oxidation of thiol to disulfide.

Volatile sulfides (predominantly $H_2S$) liberated when milk is heated have long been recognized as responsible for cooked flavor. Doubtless, these originate, at least in part, by β-elimination from Cys residues of fat globule membrane proteins and possibly β-lactoglobulin. Heating skim milk momentarily to 90°C and then aspirating for 30 min releases about 0.16 mg (0.005 mmol) of volatile sulfur per liter. Heating cream gives much higher

quantities (e g , > 1 mg·liter$^{-1}$ for 25% fat cream) Milk contains 3 2 g (0 18 mmol) of β-lactoglobulin per liter (Table 6 3), and the quantity of Cys residues in the fat globule membrane proteins is smaller Thus, the volatile sulfur released under these conditions is but a small part of the total thiol and disulfide of the protein

Potentially, thiols react with dehydroalanine residues to form lanthionine cross-links (reaction 9), but analyses to date indicate only very small amounts of lanthionine in 1% β-lactoglobulin solution heated at pH 6 9 for 80 min at 97°C However, heating "dry" casein for 27 h at 115°C and "dry" lactalbumin for 54 h at 115°C resulted in the formation of 2 7 and 12 0 mg lanthionine per gram of protein, respectively

The ε-amino group of lysyl residues of proteins participates in the Maillard reaction with carbonyls (Section 10 4) and also can react with dehydroalanine and carboxyl side chains to form cross-links (reactions 10, 11, and 12) These latter reactions occur only under rather drastic conditions of heating The isopeptides, ε-N(β-aspartyl)lysine and ε-N(γ-glutamyl)lysine, have been detected in "dry" casein or lactalbumin heated at 112°C but not in milk products Lysinoalanine is formed particularly during heating under alkaline conditions It may amount to 100–500 mg per kilogram of protein of sterilized whole or evaporated pasteurized or UHT milk, and in some milk protein preparations (particularly caseinates) and derived products higher quantities have been found This has caused some concern, because high doses of lysinoalanine are toxic to rats under some conditions It is, however, extremely unlikely that comparable quantities are to any extent toxic to humans

## 10.4 BROWNING REACTIONS

Nonenzymatic browning of food products results from degradation of sugars and interactions of the degradation products Heat-induced browning reactions of sugars in the absence of amino compounds are generally called caramelization They involve enolizations and dehydrations catalyzed by acids or bases A second type of reaction leading to browning occurs when amino compounds as well as sugars are present These are called sugar–amine or Maillard reactions Amino acids, peptides, or proteins condense with the sugar and act as their own catalysts for enolization and dehydration The degradation of the sugar follows much the same course as in caramelization, but the reactions occur under milder heat treatments and at pH near neutrality The Maillard reaction pathway is the important one in milk and dairy products

### 10.4.1. Chemistry of Maillard Reactions

The primary reaction in Maillard browning is condensation of an amino compound with the carbonyl group of a sugar in the open-chain form, presumably to form a Schiff base although such a compound is not isolatable

An Amadori rearrangement occurs with the formation of a N-substituted 1-amino-1-deoxy-2-ketose, which can be isolated and identified. These compounds are colorless and do not absorb light in the near ultraviolet. The 1-amino-1-deoxy-2-ketose enolizes to either a 1,2-eneaminol or a 2,3-enediol; a pathway of degradation stems from each of them. The former gives rise to 5-hydroxymethyl-furfural (HMF) and the latter to a methyl α-dicarbonyl intermediate, which, with various rearrangements, can lead to maltol, C-methyl reductones and α-dicarbonyls such as 2-oxo-propanal (also designated methylglyoxal or pyruvaldehyde), acetol, diacetyl, and hydroxydiacetyl. A third pathway arises from the so-called Strecker degradation, which involves reaction of a dicarbonyl compound with an amino acid to yield an aldehyde containing one less C than the amino acid, $CO_2$, and pyrazines. Figures 10.6 and 10.7 give a summary of these reactions.

Some of the compounds formed by the Maillard reactions are reductones having an enediol conjugated with a carbonyl function. This is the same structure that occurs naturally in L-ascorbic acid; such compounds are powerful reductants in acid solution. Reaction of some of the carbonyl compounds with amines leads to polymers called melanoidins. These are brown compounds of variable structure and solubility. They have unsaturated heterocyclic rings, which account for their absorption of ultraviolet light and their fluorescence. Some of the products of the Maillard reactions, such as maltol, are somewhat volatile and contribute to caramelized flavor in heated products. (See Section 18.3.2.) The brown pigments and some of the other products are strongly (covalently) bound to protein, but the chemistry of their attachment has not been defined clearly.

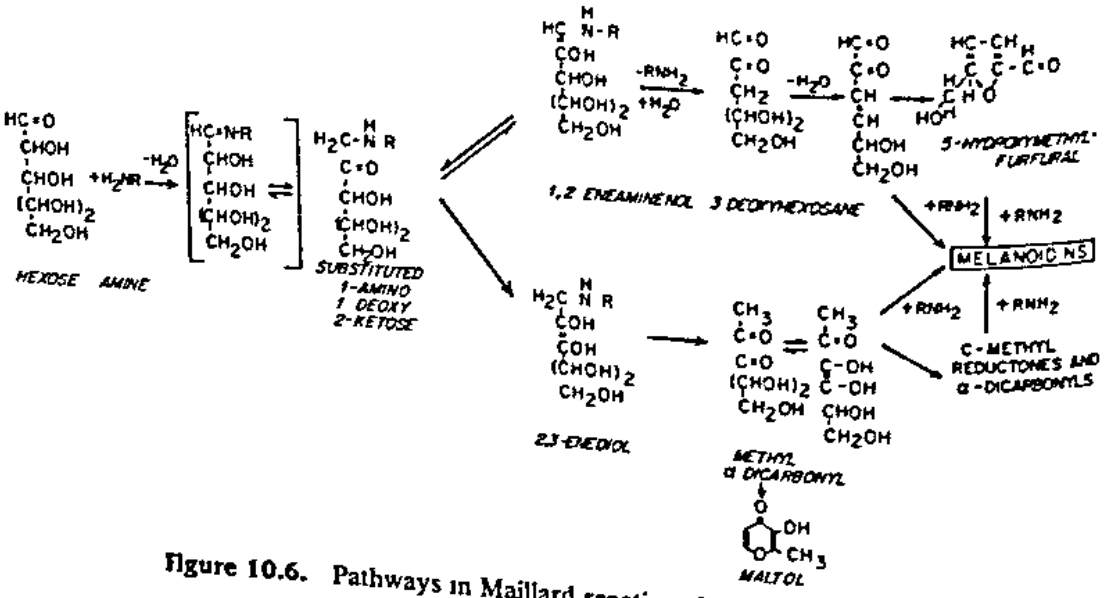



Figure 10.6. Pathways in Maillard reactions leading to browning.

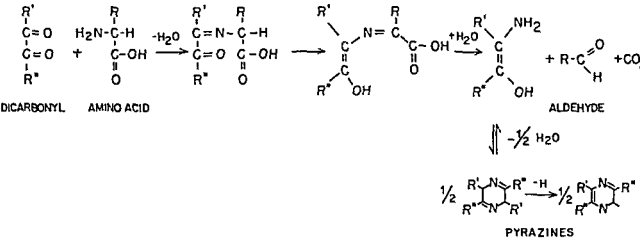



**Figure 10.7.** Pathway of Strecker degradation.

## 10.4.2. Browning Reactions in Milk and Dairy Products

In milk the principal potential Maillard reactants are lactose and the lysine residues of the proteins. Whey freed of protein by heating at 80°C and pH 5.5 browns only slightly on subsequent heating at 120°C and pH 5.5–7.5. (See Figure 10.8.) Lactose-free skim milk, prepared by first removing solutes of low molecular weight by gel filtration and then adding back a salt mixture, does not brown upon heating. Its failure to do so cannot be attributed solely to lack of lactose, however, because certain other solutes (e.g., monosaccharides, ascorbate, urea, and sugar phosphates) also are missing. Thus, the possibility of participation of some highly active reactants present in low concentrations has not been excluded rigorously. Both whey and skim milk are browned by intense heat treatments.

Manifestations of the browning reactions that affect the utility of dairy products, or that are useful in monitoring the course of the reactions, are production of compounds, brown color, fluorescence, off-flavor, increase in reducing power measured at pH 6.5 or below, and decrease in available lysine.

Many compounds have been isolated and identified from extracts of "dry" systems of milk proteins and lactose and dry milk and dry whey incubated

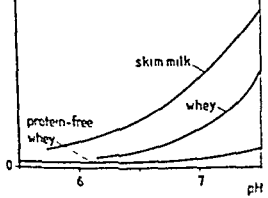



**Figure 10.8.** The brown color intensity (*B*, arbitrary units) developed in various milk fractions at different pH as a result of heating at 120°C for 20 min. After S. Patton, *J. Dairy Sci.* 35 1952):2053.

at pH 6–7 and water activity of 0.75–0.80. Most of these compounds could have originated from the reactions of the Maillard and Strecker pathways and subsequent interactions. Prominent among them are 5-hydroxymethylfurfural, furfuryl alcohol, furfural, maltol, acetol, 2-oxo-propanal, acetaldehyde, and formic, acetic, propionic, butyric, and lactic acids.

5-hydroxymethyl-furfural is measured conveniently by a colorimetric reaction and has been used considerably for monitoring the course of its reaction sequence. Maltol and some of the other compounds contribute to the flavor of dairy products.

Brown color is undesirable in most dairy products, except that some users prefer concentrated milk used in coffee to have a light tan color. The intensity of brownness can be assessed by measurements of reflectance of the product (Figure 11.3) or by absorbance of a tryptic digest. Some of the compounds formed absorb ultraviolet light and emit fluorescence in the blue and yellow regions of the spectrum. These compounds are extractable by ether and thus can be differentiated from riboflavin, the prominent water-soluble fluorophor of milk.

Palatability is impaired long before actual browning is detectable. Thus, the compounds formed in the earlier colorless stages of the reactions are of most interest. Such compounds and their flavor properties are described in Section 18.3.2.

The capacity of reductones to reduce ferricyanide at pHs below neutrality has been used to assess the extent of the browning reactions during manufacture and storage of dry milk products. Determination of the reducing capacity at pH 5.6 of protein precipitated from the product eliminates ascorbate as a reductant. This so-called protein acid ferricyanide reducing substance (protein-AFRS) is a sensitive measure of the extent of the Maillard sequence.

Many of the reaction products of lysine with carbonyl compounds are not digestible by humans and animals, and consequently the Maillard reaction renders lysine unavailable. The loss in nutritive value of the protein from this cause is not serious in most milk products. Drastic heating of concentrates or storage of dried products at high temperatures and high humidities are required to impair the availability of lysine seriously.

The rates at which the several manifestations of the Maillard reactions occur in dairy products are highly dependent on pH, time, temperature, and water activity. The effect of pH on browning is illustrated for skim milk and whey in Figure 10.8. Because acid is produced by the reactions (see Figure 10.1), they are self-inhibiting to a degree.

The interaction of time and temperature in promoting the browning reactions is depicted in Figure 10.9. The rate of these reactions increases with increase in concentration of solids in concentrated milk products, reaches a maximum at a water activity ($a_w$) of about 0.6 (approximately 10% water in "dry" products), and declines at lower values of $a_w$. (See Figure 17.5.) Under a given set of conditions monosaccharides react more rapidly with

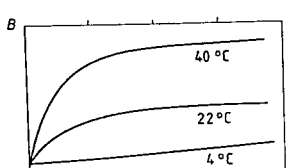



**Figure 10 9** The brown color intensity (*B* ar bitrary units) developed in evaporated milk during storage at various temperatures After S Patton *J Dairy Sci* 35 1952) 1053

amino compounds than disaccharides Thus, a lactase treatment to hydrolyze lactose increases the susceptibility of products to the browning reactions

## 10.5. FOULING

Fouling, or the deposition of a layer of milk components on a surface, often occurs It causes problems in cleaning because the deposited layer usually cannot be removed by simple rinsing with water A simple case of fouling is the drying up of a film of (diluted) milk left on a surface But there are other more specific cases of fouling by milk and milk products, which also cause specific problems

1. Fouling may occur on the metal surface of a heat exchanger This causes the heat transfer through the surface to diminish and the re sistance to flow of the milk through the heat exchanger to increase Eventually, the apparatus ceases to function
2. During ultrafiltration and reverse osmosis of milk products (Section 17 6) a layer is deposited on the membrane (at the pressurized side) that causes a considerable reduction in the flux through the membrane
3. During heating of milk in an open vat a skin is formed at the air–water interface This is a nuisance because the skin is insoluble

The following discussion deals with some general aspects of fouling, fol lowed by a more detailed consideration of problem 1 above

Fouling always starts with adsorption (See also Section 12 1 2 ) Milk and its derivatives contain many surface-active substances, for instance, polar lipids and proteins The adsorption will depend on the nature of the surface and the composition of the product, but in practice it is almost always protein that adsorbs (even if the product is an ultrafiltration permeate, which still contains some low molecular weight peptides) Proteins adsorb at hydrophobic surfaces (e g , most plastics air) by hydrophobic bonding, usually the protein molecules change their conformation on adsorption At hydro-

philic surfaces (glass and most metals, if clean), electrostatic bonds are involved, and the presence of divalent cations ($Ca^{2+}$, $Mg^{2+}$) may increase the bond strength greatly. The serum proteins are usually the ones adsorbed. Casein micelles (or the paracasein micelles formed by renneting) are not adsorbed onto hydrophobic and some metallic surfaces (e.g., copper). They are adsorbed onto hydrophilic surfaces; consequently, the gel formed after renneting or souring of milk sticks to the surface; this happens with (clean) glass or with stainless steel rinsed with alkaline liquids. Giving the metal surface a small electrostatic charge suffices to prevent adsorption of casein micelles, though not of globular proteins. Some bacteria can stick to a variety of surfaces by means of their glycocalix, which consists of polysaccharide chains that protrude from the cell wall and have end groups that bind to various surfaces.

Adsorption is not the cause of fouling. Adsorption occurs because it lowers the interfacial free energy and is thus restricted to a monomolecular (or monoparticulate) layer. Moreover, adsorption always occurs, and fouling occurs only under restricted conditions. But adsorption causes a change of the surface, and onto this changed surface any further deposition occurs. In the case of membranes, adsorption of a monomolecular protein layer may cause some reduction of the flux, but additional deposition of material, causing further reduction of flux, commonly occurs.

Deposition may result from a local supersaturation or from reactions occurring specifically or faster at the surface, or from both. The concentration of a solute may be higher near the surface; this occurs near a membrane in membrane processes because of concentration polarization (the increase in solute concentration near the membrane caused by the local solvent flow) and near the milk–air interface because of evaporation. The temperature may be higher or lower near the surface, as occurs in heat exchangers, but fouling by milk products predominantly occurs at a heating surface. Milk is supersaturated with respect to calcium phosphate (Section 4.4), and its solubility decreases with increasing temperature. Apart from the skin on heated milk (which contains mostly protein and fat) deposits formed by fouling always contain relatively much Ca and phosphate. Any temperature-induced reaction of a protein will occur faster near a heated surface. Combination of local precipitation of calcium phosphate and local reactions causing insolubilization of protein appear to be at the root of most of the fouling caused by milk and milk products.

Some factors complicate this simple picture. Nucleation is essential in any precipitation; it should happen at the surface rather than throughout the liquid for fouling to occur. In fouling of heat exchangers, an induction period (time lag) often is observed, and this may be a result of the time it takes for sufficient nucleation to occur at the heated surface. Fouling of membranes shows no appreciable induction period. Local concentration and local temperature depend on the rate of flow along the surface, hence on total flow

rate and turbulence. These variables thus may strongly affect fouling rate, the rate decreasing with increasing flow rate.

The deposits forming on a heated surface are usually classified into two types. Type A occurs typically at moderate temperatures (say 85°C); it contains, for instance, 30–35% "ash" and 50–60% protein in the dry matter, as well as a little fat; it looks soft, voluminous, and curdlike, and it has a creamy color. Type B occurs typically at higher temperatures (say > 100°C); it contains more than 70% "ash" in the dry matter (mostly tricalcium phosphate), as well as protein and a little fat; it looks compact, gritty, and grayish, and it is also called milk stone or, more generally, scale. The deposit on the membrane when whey is ultrafiltered is comparable to type B in composition. At high temperatures scale also may be deposited on a nonheated (though usually not on a cooled) surface; in accordance with this observation its rate of formation depends little on flow rate. Also, if hardly any protein is present (e.g., when heating an ultrafiltration permeate) a scale is deposited, but it is removed easily by rinsing with weak acids. At higher pH, more milk stone usually is formed, presumably because of the decreased solubility of calcium phosphate.

In the formation of a type A deposit, reactions of the proteins, particularly interchanges of thiol groups and –S–S– linkages, are involved. Rapid oxidation of thiol groups by $KIO_3$ markedly diminishes fouling. Heating at such a high temperature that the whey proteins are rapidly denatured causes less fouling of this type (but more of type B). Nevertheless, whey protein denaturation at the heated surface is not the only or even the main mechanism. The temperature dependence of fouling is far smaller than that of heat denaturation; this may be caused by the fact that at higher temperatures the denaturation reactions take place in the bulk rather than at the surface. But a solution devoid of whey protein, such as sodium caseinate in ultrafiltration permeate, also shows fouling, albeit at a slower rate than skim milk or whey. $Ca^{2+}$ ions are essential, and an increase in their activity strongly increases fouling rate. Whey that has been freed of Ca shows no fouling. Normal deposits are rich in calcium phosphate, and it may well be that linkages such as those in casein micelles are involved. The fouling mechanism clearly is intricate there may be some similarity with heat coagulation. (See Section 13.5.)

Besides heating temperature, temperature difference between heating surface and milk, and flow conditions (all of which affect local temperature), several factors influence fouling rate, but little quantitative information can be given:

1. The lower the pH, the more fouling. Milk turned slightly sour by bacterial action (say, to pH 6.4) shows copious fouling.
2. There are large differences among lots of milk, for instance, with season. Several variables may be involved: pH, content of phospho-

lipids (they tend to decrease fouling), content of free fatty acids (capric acid tends to diminish, stearic acid to increase fouling); $Ca^{2+}$ activity and possibly phosphate content (more phosphate giving less deposit, but one that is more difficult to remove), and perhaps concentration of thiol groups.

3. Cold storage of milk (up to 20 h) reduces the deposit. The explanation is not clear.
4. Preheating the milk to such an intensity that whey proteins are denatured reduces (but does not eliminate) type A fouling. Preheating reduces fouling in ultrafiltration of whey, presumably because of the resulting decrease in dissolved calcium phosphate.
5. Concentrated milk shows much quicker fouling than plain milk, as is to be expected.
6. The formation of air bubbles at the heated surface markedly increases fouling. It may be that surface denaturation of proteins is involved, but it is more likely that the cause is a higher surface temperature near the bubbles. Degassing of milk diminishes fouling.

As stated above, fouling causes a cleaning problem. The deposit allows copious bacterial growth, so disinfection cannot be achieved in practice without removing it, which is not easy. The protein can be dissolved in hot alkaline solutions. The calcium phosphate dissolves in weak acids or in a solution of a calcium-chelating agent like sodium ethylenediaminetetraacetate. To remove lipids and to keep the soaked-off deposit in suspension, a detergent (i.e., a suitable surface-active agent) is needed. Modern cleaning agents usually combine these active principles.

## *Suggested Literature*

Kinetic aspects of heat-induced changes in foods are discussed in a series of articles in

*Food Technology* (February 1980) 51–88.

Reaction kinetics in general are treated in textbooks on physical chemistry. Reactions of proteins during heating are discussed by

J. C. Cheftel, Chapter 6 in S. R. Tannenbaum, ed., *Nutritional and Safety Aspects of Food Processing* (New York: Dekker, 1979).

An extensive symposium report on this topic is

M. Friedman, ed., *Protein Crosslinking*, vols. A and B (New York: Plenum Press, 1977).

This also contains an article on milk proteins by H. Klostermeyer and E. H. Reimerdes, vol. B, pp. 263–75.

Chemical aspects of Maillard reactions are reviewed extensively by

T. M. Reynolds, *Adv. Food Res.* 12 (1963) 1, and 14 (1965): 168.

And the nutritional and physiological consequences by

J Adrian, *World Rev Nutr Diet* **19** (1974) 71

A now somewhat outdated review on Maillard reactions in milk is

S Patton, *J Dairy Sci* **38** (1955) 457

A review on deposit formation from milk in heat exchangers is by

H Burton, *J Dairy Res* **35** (1968) 317–30

Several aspects of fouling are discussed in the proceedings of a conference on

"Fundamentals and Application of Surface Phenomena Associated with Fouling and Cleaning in Food Processing," University of Lund, Sweden, 1981

# 11

# Some Properties

In this chapter we discuss some physical and chemical properties and reactions of milk and milk products that cannot be handled conveniently under one of the other chapters. Additional physical properties are tabulated in the appendix.

## 11.1. DENSITY

The density (more precisely, mass density or volumic mass) is the mass of a certain quantity of a material divided by its volume. It is thus expressed in $kg \cdot m^{-3}$ (SI units) or $g \cdot ml^{-1}$ (c.g.s. units); these differ by a factor of 1000 (or, more exactly, 999.972). The symbol is $\rho$, and because density closely depends on temperature it is usually denoted with temperature, $\rho^{20}$ meaning the density at 20°C. Another quantity is the specific gravity or specific weight (s.g.), the mass (or weight) of a certain volume of the product divided by the mass of an equal volume of water. Thus, s.g. $= \rho_{product}/\rho_{water}$, a dimensionless quantity. Two temperatures are needed to define its value, and we have, for instance, $s.g.{}^{20}_{20}$ or $s.g.{}^{20}_{4}$. The latter almost equals $\rho^{20}$ in c.g.s. units because $\rho^4$ of water equals $1.000\ g \cdot ml^{-1}$. The density of a liquid product is used to convert volume into mass and vice versa. (It should be taken into account that the mass obtained by weighing has to be corrected for the difference in buoyancy of product and weights in air; for $\rho \approx 10^3\ kg \cdot m^{-3}$, room temperature, and brass weights, the correction is $+0.1\%$.) Density occurs in several equations on physical properties. The density of milk also is used in formulas to calculate its total-solids content. (See Section 20.2.)

$\rho^{20}$ of milk is about $1030\ kg \cdot m^{-3}$, but it depends on composition. The density of a mixture of components like milk can be derived by summation over all of its components according to

$$\frac{1}{\rho} = \sum \left(\frac{m_x}{\rho_x}\right) \qquad (11\ 1)$$

where $m_x$ is the mass fraction of component x, and $\rho_x$ its apparent density in the mixture $\rho_x$ usually is not the density of the pure substance, because a change in volume (usually contraction) occurs when two components are mixed Taking arbitrarily for $\rho^{20}_{water}$ the density of pure water (i e , 998 2 kg m$^{-3}$), we find for $\rho^{20}$ of fat about 918, for protein about 1400, for lactose about 1780 and for the residual components of milk about 1850 kg m$^{-3}$ With these data $\rho^{20}$ of milk products can be calculated, some data are in the appendix (See Table A 22 )

The density of milk is rather variable (for the most part $\rho^{20}$ is between 1027 and 1033 kg m$^{-3}$), and the main cause is variation in fat content ($m_F$) On average, the density of skim milk, milk, and cream (in kg m$^{-3}$) is given by

$$\rho^{20} \approx \frac{1000}{0\ 123\ m_F + 0\ 9665} \qquad (11\ 2)$$

The density of milk products concentrated by removing water can be calculated by a combination of Equation 11 1 for the initial and the concentrated product Some rearrangement leads to

$$\frac{1}{\rho_c} = \frac{R}{\rho_o} + \frac{1 - R}{\rho_w} \qquad (11\ 3)$$

where $R$ is the concentration factor (ratio of dry matter content in the concentrated milk to that in the unconcentrated milk) and subscripts o, c, and w denote initial milk, concentrated milk, and water, respectively

The density of milk decreases with increasing temperature (appendix, Table A 22) mainly because water expands Specific gravity is nearly constant, a small decrease with increasing temperature is caused by the expansion of fat being larger than that of water and the contraction on mixing of the other solids with water slightly decreasing Part of the fat solidifies on cooling, and this causes a further increase in density The contraction of milk fat for complete solidification is about 70 cm$^3$ kg$^{-1}$, but only a small part solidifies at room temperature (See Section 5 7 ) Moreover, this depends on temperature history, considerable supercooling can occur Liquid milk fat at 20°C will have a density of about 915 kg m$^{-3}$, and if crystallization equilibrium at that temperature is reached, $\rho^{20}$ will equal about 928 kg m$^{-3}$ Most density measurements are made directly after the milk has been prewarmed to 40–45°C and cooled again to 20°C, so the fat is still almost fully liquid during testing, hence $\rho^{20} \approx 915$ (The value of 918 taken above for the apparent density takes into account the higher density of the milk fat globule membrane ) The differences caused by temperature history may become appreciable, particularly for cream A 20% fat cream has, for example, $\rho^{20}$ ~ 1009 kg m$^{-3}$ if measured with the fat in supercooled state, but if the cream

has been kept for some hours at 4°C and subsequently warmed to 20°C, we would find $\rho^{20} \approx 1019$ kg·m$^{-3}$.

A further complication is inclusion of air, which may readily happen in viscous products. It considerably lowers density, and density also becomes appreciably dependent on pressure.

## 11.2. OPTICAL PROPERTIES

The *refractive index* (common symbol $n$) of a transparent medium is usually defined as the ratio of the velocity of light in air to that in the medium (or the ratio of the wavelength of light in air to that in the medium). The refractive index is characteristic for a particular material, but it also depends closely on temperature (and pressure) and on the wavelength of the light ($\lambda$); usually, $n$ decreases with increasing $\lambda$. It is often measured at $\lambda = 589.3$ nm (i.e., the sodium D line) and 20°C, denoted as $n_D^{20}$. The dependence on temperature and pressure parallels that of density, and the term $(n^2 - 1)/(n + 0.4)\rho$ is usually almost constant for any wavelength.

The refractive index of milk ($n_D^{20} \approx 1.338$) is determined by that of water ($n_D^{20} = 1.3330$) and the dissolved substances. Particles larger than about a quarter wavelength (measured in the solution) do not contribute to $n$; this implies particles greater than about 0.1 $\mu$m for visible light. Consequently, fat globules, air bubbles, or lactose crystals do not affect $n$ of milk products. But casein micelles do, even those that are comparatively large, presumably because they are inhomogeneous and have no sharp boundary.

The difference in refractive index between pure solvent and a solution, $\Delta n$, is proportional to the volume concentration of solute. The differences caused by various solutes are approximately additive, and we thus have for the solution

$$n(\text{solution}) - n(\text{solvent}) = \Delta n = \rho \sum m\, r \qquad (11.4)$$

where $\rho$ is density of the solution, $m$ is mass fraction of a solute, and $r$ is its specific refraction increment. The value of $r$ of a solute is usually fairly independent of concentration and other conditions. The volume contraction resulting when mixing solute and solvent is included in $r$, which implies that $r$ depends on the solvent and somewhat on temperature. Values for $r$ in water at $\lambda = 589.3$ nm and 20°C are, in ml·g$^{-1}$,

Casein micelles, 0.207 (expressed per gram of dry casein)
Serum proteins, 0.187
Lactose, 0.140
Other dissolved substances, ~ 0.170
Sucrose, 0.141

With these data, the refractive index of milk products can be calculated. When concentrating milk or whey by evaporation, $\Delta n/\rho$ increases propor-

tionally with the concentration factor ($R$) as long as no crystallization of lactose occurs, the effect on $n$ of an increase in colloidal calcium phosphate appears to be very small Refractive index can be determined easily, rapidly, and with considerable accuracy ($\sim 10^{-4}$ or better), so it is often a useful parameter to check changes in composition

*Light scattering* is caused by particles of larger than molecular dimensions whose refractive index differs from that of the surrounding medium In milk the fat globules and the casein micelles are primarily responsible, scattering by serum protein molecules and by somatic cells is almost negligible because of their small size and low concentration, respectively

The particles scatter the incident light into every direction (See Figure 11 1 ) We can measure the scattered intensity at some angle from the incident beam (e g , 90°), and such measurements often are made on suspensions of particles much smaller than the wavelength ($\lambda$), the intensity then is about proportional to the square of the particle volume, the square of the difference in refractive index, and $\lambda^{-4}$ Another measurement is that of turbidity (i e , the attenuation of the incident beam caused by scattering), usually expressed as absorbance or optical density The turbidity is affected by the same variables, but because the technique is used for larger particles the relations are more complicated

Fat globules are fairly large spherical particles, and if their concentration is low enough turbidity can be derived fairly easily from theory For visible light the absorbancy $A$ is approximately given by

$$A = 0\,5\,\tau\phi\left(\frac{l}{\lambda}\right) \qquad (11\,5)$$

where $\phi$ is the fat volume fraction and $l$ is the optical path length The specific turbidity $\tau$ is a function of $d/\lambda$ (where $d$ is globule diameter) and is shown in Figure 11 2 To some extent this is an oversimplification The turbidity depends somewhat on the instrumental setup The refractive index difference between globule and plasma ($\sim$ 0 12–0 14) affects turbidity and

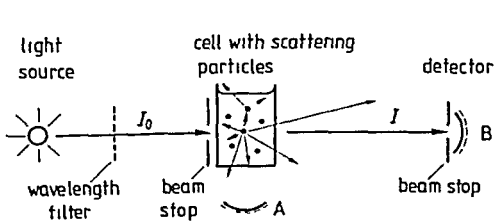



Figure 11.1. Light scattering measurements Detector A measures scattered light detector B measures turbidity (log $I_0/I$ where $I_0$ means intensity of the unscattered light) Multiple scattering is indicated by broken arrows

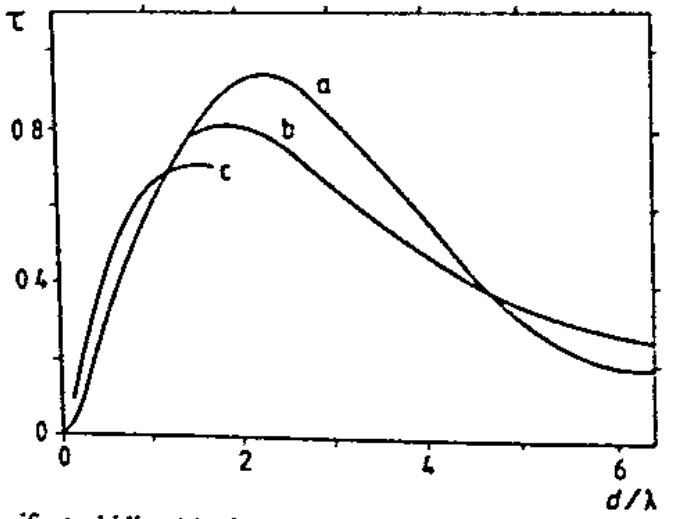



**Figure 11.2.** Specific turbidity ($\tau$) of milk fat globules in water as a function of globule size ($d$) to wavelength ($\lambda$). (a) Curve for fat globules of uniform size; (b) results for a sample of whole milk; (c) homogenized milk. Approximate examples. After P. Walstra, *Neth. Milk Dairy J.* 19 (1965):93.

varies with $\lambda$. Moreover, the refractive index of milk fat varies among lots of milk; approximately $n_D^{40} = 1.452–1.457$. The fat globules are not all of the same size, and this causes the turbidity curve to broaden and flatten, the more so as the polydispersity is more pronounced. Figure 11.2 gives some examples, and now $d$ means an average globule diameter (in this case $d_{vs}$—see Section 12.4). According to equation 11.5 and Figure 11.2, turbidity can be used to estimate $\phi$, hence fat content, if conditions are chosen so that $\tau$ is almost constant with average $d$ (i.e., near the maximum in the curve). Variations in polydispersity and in refractive index of the fat still cause deviations. On the other hand turbidity measurement, particularly at a range of wavelengths, is a powerful and easy tool to estimate average globule size and polydispersity.

Casein micelles are smaller than fat globules ($d/\lambda \approx 0.02–0.50$) and scatter less light. This also implies that the scattering intensity strongly increases with decreasing wavelength (blue light is scattered far more than red). The scattering data are very difficult to interpret, because of the inhomogeneity of the casein micelles. Their scattering greatly increases with their content of colloidal calcium phosphate.

For concentrated dispersions like undiluted milk, scattering theory is far more intricate. If the interparticle distance is on the order of a wavelength or smaller, scattering is weakened by negative interference (dependent scattering). Another complication is multiple scattering; light scattered by one particle reaches another one and is scattered again, and so on. (See Figure 11.1.) In undiluted milk, multiple means, for instance, 1000 times or more.

*Absorption* of visible light by milk is weak. Milk serum contains riboflavin (see Figure 11.7 for its formula), which gives whey a yellow-green color. The maximum absorbance is near $\lambda = 0.47$ $\mu$m, specific extinction of serum

about 0.05 $cm^{-1}$ (i.e., for an optical path length of 1 cm the absorbance is 0.05). Part of the absorbed energy is emitted as fluorescent light, $\lambda \approx 0.53$ $\mu$m. Milk fat contains carotenoids, primarily $\beta$-carotene, which is responsible for its yellow color. Maximum absorbance is near $\lambda = 0.46$ $\mu$m, specific extinction (e.g., 3 $cm^{-1}$) but widely variable.

In the ultraviolet, the aromatic rings of the proteins (in tyrosine and tryptophan residues) strongly absorb near $\lambda = 0.28$ $\mu$m, and double bonds of the fat absorb very strongly near $\lambda = 0.22$ $\mu$m. In the infrared, numerous strong water absorption bands occur for $\lambda > 1.2$ $\mu$m (except near $\lambda = 1.7$ $\mu$m), making milk very opaque to this radiation. Some other strong bands are $CH_2$ near 3.45 $\mu$m (mainly lipids); aliphatic ester bonds near 5.70 $\mu$m (mainly lipids); peptide bonds near 6.50 $\mu$m (mainly proteins), undissociated COOH groups near 5.90 $\mu$m, and OH groups near 3.50 and 9.60 $\mu$m (mainly lactose), but there are numerous others. These absorptions can be used to estimate the contents of the major components of milk.

A *diffuse reflection* spectrum of milk is shown in Figure 11.3. It is obtained by measuring light scattered in various directions from a surface, excluding the direction of truly reflected incident light (mirror reflection); a purely white surface is taken as a reference (100%). The results (in particular the overall level of the curves) closely depend on the experimental arrangement. The diffuse reflection of a liquid like milk results from multiple scattering by the particles in a surface layer of a certain thickness (often some millimeters). The optical path in the milk may be considerable, at least for part of the light, and some light can thus be absorbed. This is seen in Figure 11.3, where the minimum in the reflection curve near $\lambda = 0.46$ $\mu$m is caused by absorption, particularly in the $\beta$-carotene in the fat. It gives milk its creamy color. Compare also the reflection by milk and by skim milk in Table 11.1. Skim milk scatters less light because it has fewer particles, but at short wavelength its reflection is higher than that of milk because of the absence of absorption by $\beta$-carotene. Moreover, the casein micelles scatter blue light (short wavelength) more effectively than red, hence the bluish color of skim milk. The riboflavin in skim milk also causes a little absorption near $\lambda =$

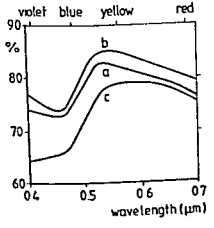



**Figure 11.3.** Examples of diffuse reflection (%) of homogenized milk as a function of wavelength. (a) Unheated; (b) heated for 30 s at 130°C; (c) heated for 40 min at 115°C. After H. Radema, Netherlands Institute for Dairy Research (unpublished).

**Table 11.1. Diffuse Reflection by and Penetration of Light into Milk Products**

| Parameter Measured | Wavelength (nm) | Skim Milk | Whole Milk | Homogenized Milk |
|---|---|---|---|---|
| Diffuse reflection (%) | 450 | 48 | 43 | 52 |
| | 550 | 49 | 53 | 63 |
| | 750 | 32 | 46 | 58 |
| Penetration (mm) | | | | |
| to 10% intensity | 440 | 5 | 3 | 2 |
| | 580 | 14 | 8 | 5 |
| to 1% intensity | 440 | 13 | 7 | 6 |
| | 580 | 36 | 24 | 18 |

*Note:* Approximate examples from various sources.

0.45 $\mu$m. It is further seen in Table 11.1 that homogenization of milk causes the diffuse reflection to increase, thus making the milk whiter. The differences may seem small, but the eye is a very sensitive instrument for slight color variations.

Heat treatment of milk causes color changes. (See Figure 11.3.) At first the milk becomes slightly whiter; this is usually ascribed to denaturation of serum proteins, leading to more scattering particles, but it is more likely that an increase in colloidal phosphate content of the casein micelles is responsible. More intense heating causes Maillard reactions (Section 10.4) and the ensuing brown pigments markedly alter the reflection spectrum, particularly at short wavelengths. In practice, we usually assess the browning caused by heat treatment by visual comparison with color standards.

The *penetration of light* into milk products may be important with respect to light-induced reactions. Any light falling onto a turbid liquid will either emerge again (diffuse reflection) or be absorbed. The stronger the scattering and the absorption, the smaller the penetration depth; some examples are in Table 11.1. Penetration is defined here as the distance into the liquid after which the light intensity is reduced by a certain factor. Also, this quantity closely depends on experimental conditions.

## 11.3. ACIDITY

The acidity of a solution usually is expressed as $pH = -\log a_{H^+}$, where $a_{H^+}$ is the $H^+$ ion activity in moles per liter. The pH of most samples of milk is 6.6–6.8, average about 6.7 at 20°C (or 6.6 at 25°C). This implies that $a_{H^+}$ ranges from 0.16–0.25 $\mu M$. The pH of milk generally increases with advancing lactation; that of colostrum can be as low as 6.0. The pH of most mastitic milk is high, up to 7.5.

The acidity of a solution results from the ionization of groups that can split off or bind protons ($H^+$ ions). Properties of ions and aspects of ionization are discussed in Section 4.2.1. Consider an acid HA that is in equilibrium with $H^+ + A^-$; repeating Equation 4.8, we have for the ionization

$$\log \left[\frac{[A^-]}{[HA]}\right] = pH - pK \qquad (11.6)$$

where [ ] means molar concentration, $pK = -\log K_a$, and $K_a$ is the dissociation constant. If pH << p$K$, the acid group is undissociated, if pH >> p$K$ it is dissociated (ionized). More precisely, if pH = p$K$ the group is 50% ionized; if pH − p$K$ = ± 1, ionization is 50 ± 41% complete. An acid is said to be strong if its p$K$ is low (its $K_a$ large). To be sure, in equation 11.6 activities rather than concentrations should be used; only for zero ionic strength is ion concentration equal to ion activity. For the ionic strength of milk and for a monoprotic acid, the stoichiometric p$K$ (i.e., the pH of equal concentrations of $A^-$ and HA) is about 0.1 higher than the intrinsic p$K$ that is given in reference books. Basic groups, like $-NH_2$, can be treated in the same way; at pH below p$K$ the group is ionized to $-NH_3^+$ and is thus an acid (it can donate protons), at pH >> p$K$ it is not ionized. A base is said to be strong if its p$K$ is high (its $K_a$ small).

Milk contains many acidic and basic groups. This is reflected in a titration curve as given in Figure 11.4; milk is titrated with respect to pH by either a strong acid or a strong base (i.e., an acid or base that is fully dissociated over the pH range considered). Because of the presence of acidic and basic groups, the pH changes much less than when titrating water; these groups

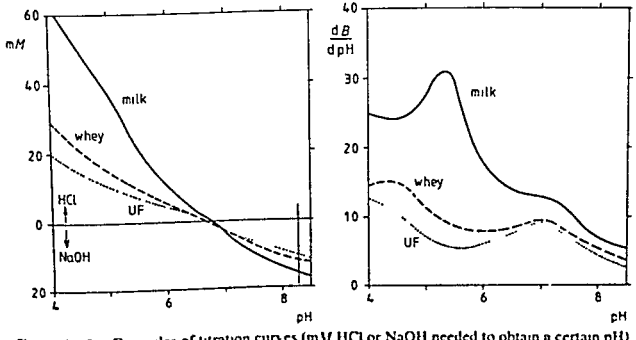



**Figure 11.4.** Examples of titration curves (mM HCl or NaOH needed to obtain a certain pH) of milk and of sweet whey and ultrafiltrate made from that milk; the same results also expressed as buffer index $dB/d$ pH (in mmol liter$^{-1}$). Newly determined.

have a buffering action. The buffering capacity of a solution can be expressed in the buffer index, which is the molar quantity of acid or base needed to change the pH by one unit; it is thus the derivative of the titration curve. The buffer index of an ionizable group is given by

$$dB/dpH \approx 2.3\ c\ K_a \frac{a_{H^+}}{(K_a + a_{H^+})^2} \qquad (11.7)$$

where $C$ is the total (dissociated plus associated) molar concentration of the group. Again, it concerns intrinsic constants, but equation 11.7 also holds for monoprotic acids if $K_a$ is the stoichiometric dissociation constant. Equation 11.7 implies that the buffering capacity is maximal (0.58 $c$) for pH = p$K$; it is reduced to 33% of the maximal value for pH − p$K$ = ± 1, and to 4% for pH − p$K$ = ± 2.

The buffering groups of milk are given in Table 11.2. Compare also the titration and buffer index curves of milk, whey, and ultrafiltrate of Figure 11.4. It follows that the ionizable groups of the proteins, the phosphates,

**Table 11.2. Buffering Groups in Milk**

| Group | Concentration (m$M$) | p$K$ |
|---|---|---|
| **Protein-Bound Residues** | | |
| Aspartic acid | 19 | 4.1 |
| Glutamic acid | 50 | 4.6 |
| Histidine | 6 | 6.5 |
| Tyrosine | 12 | 9.7 |
| Lysine | 20 | 10.5 |
| Ester-phosphate | 7 | 2., 6? |
| N-acetyl neuraminic acid | 0.5 | 5 |
| Terminal groups | 1.5 | 3.7, 7.9 |
| **Salts** | 21[b] | 3., 5.8, 6.6 |
| Phosphate[a] | | |
| Citrate[a] | 9 | 3., 4.1, 4.8 |
| Phosphate esters[a] | 2.5 | 1.7, 5.9 |
| Carbonate | 2 | 6.4, 10.1 |
| Various carboxylic acids | 2 | 4.8 |
| Various amines | 1.5 | 7.6? |
| Lactic acid | 50–120[c] | 3.95 |

*Note.* Approximate average concentration, and their estimated (stoichiometric) p$K$ values in milk. After various sources.

[a] p$K$'s from titration with $Ca(OH)_2$.

[b] About 10 m$M$ colloidal phosphate, 11 m$M$ in solution.

[c] In sour milk products.

and the citrates mainly determine the acidity and the buffering of milk. The concentrations of these substances can vary considerably, so the titration curves vary among samples of milk. There are, however, many complications, and the data in Table 11.2 consequently are not fully reliable. Many p$K$ values are uncertain, as the ionization of a group is affected by that of other nearby groups; this may be the case in proteins. Some ionizable groups in a protein may even be unavailable for titration, as they are buried in the hydrophobic interior of the molecule; virtually all of the ionizable groups of casein are exposed, but not all of those of the whey proteins. The presence of $Ca^{2+}$ ions strongly modifies the ionization of di- and triprotic acids, such as phosphoric and citric acids; they will behave almost as if titrated by $Ca(OH)_2$ instead of by NaOH or HCl. However, much of the calcium phosphate is not dissolved but becomes so when the pH is lowered. (See Figure 4.1.) Increasing the pH causes an increase in colloidal phosphate. The Mg, citrate, and protein present affect the composition and quantity of colloidal phosphate. Consequently, the buffer indexes of the various groups are not strictly additive.

The acid range of the titration curve is important because milk is often soured. The main change upon souring is caused by the production of lactic acid. Because it has a stoichiometric p$K$ in milk of about 3.95, it is largely dissociated at pH greater than 5 and titration down to this pH takes about equimolar quantities of lactic acid or of HCl. But bacterial fermentation may cause the production of other acids than lactic (e.g., acetic), breakdown of citrate, and hydrolysis of protein; consequently, more changes result than the mere formation of lactic acid.

The basic range of the titration curve is important because of the widespread use of the titratable acidity to characterize milk (products). The titratable acidity is the buffer capacity of milk between its own pH and the pH of color development of phenolphthalein (i.e., ~ 8.3). Various standardized methods exist, prescribing titration with NaOH of strength $M/10$, $M/9$ (Dornic), or $M/4$ (Soxhlet Henkel). The acidity usually is expressed in ml NaOH per 100 ml milk, hence in mmol·liter$^{-1}$ when $M/10$ NaOH is used; we will use the latter unit. There are other differences among methods, for instance, in the pH at the end point of the titration. Moreover, the result depends on the speed of titration, mainly because of the slow formation of undissolved $Ca_3(PO_4)_2$, which causes the release of protons and hence a decrease in pH.

The main rationale for employing titratable acidity was to detect sour milk, and in some standard methods the acidity is even expressed as percent lactic acid, though fresh milk is virtually devoid of lactic acid. The titratable acidity of most fresh milk ranges from 14–21 m$M$, average about 17, and this range makes it unsuitable as a measure of small quantities of lactic acid. The titratable acidity of fresh milk generally decreases with advancing lactation and tends to be low in mastitic milk. It is primarily a measure of the concentration of protein and phosphate. The main contributions are approximately:

| | |
|---|---|
| 2.2 per % casein | ~ 5.7 |
| 1.4 per % whey protein | ~ 0.9 |
| 0.1 per m*M* colloidal inorganic phosphate | ~ 1.0 |
| 0.7 per m*M* dissolved inorganic phosphate | ~ 7.8 |
| 1.5–2.0 for other compounds | ~1.7 |
| Total average | ~ 17.1 mmol·liter$^{-1}$ |

Addition of 0.1% lactic acid would increase the titratable acidity by 11.4 mmol·liter$^{-1}$. Thus, titratable acidity provides an easy method to follow the amount of acid formed, but otherwise it makes little sense. The pH is the essential parameter for characterizing milk acidity.

The pH of milk closely depends on temperature. (See Figure 11.5.) The decrease in pH with increasing temperature does not imply that the milk becomes more acid. It is seen that the pH of water falls at least as much; the ionization of water increases with temperature, which implies that its neutral pH ($a_{H+} = a_{OH-}$) decreases. The p*K* of each buffer in milk depends on temperature, but in various ways—some increasing, some decreasing. Different authors have reported somewhat different results on the relation between temperature and pH of milk, which may be ascribed to uncertainties in pH measurements at high temperatures, to differences in milk composition, and to changes occurring with time. Examples of the latter are the slow insolubilization of calcium phosphate, loss of $CO_2$, and denaturation of whey proteins (causing more ionizable groups to become exposed). Consequently, the pH–temperature relationship may not be completely reversible; particularly, heating above 100°C causes permanent changes. (See Section 10.1.) If a dialysate is made at different temperatures and its pH then measured at room temperature, it differs from that of the original milk. (See Figure 11.5.) Again, the changes in the amount of undissolved calcium phosphate probably are responsible for the greater part; this causes a release of protons at high

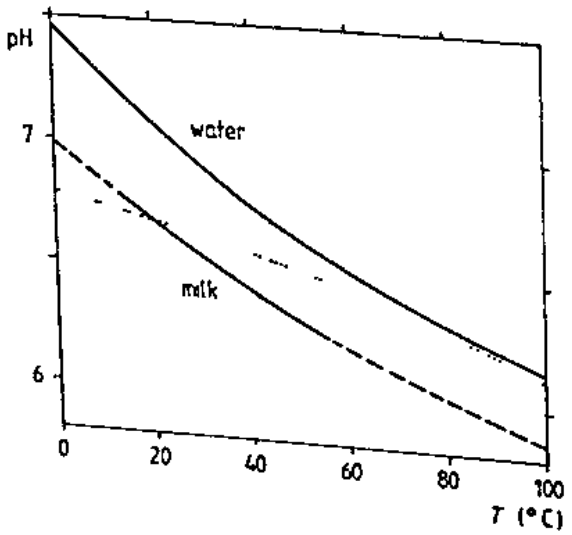



**Figure 11.5.** The pH of milk and water as a function of temperature; the broken line is less certain. The dotted line gives the approximate pH at 20°C of milk dialysate obtained at different temperatures. After various sources.

temperature, which is not reversed at room temperature because now the undissolved phosphate has been removed

Many processes cause a change in the acidity of milk Lipolysis causes the pH to decrease and the titratable acidity to increase, particularly in high-fat products Incidentally, the titratable acidity of fresh cream is lower than that of milk, because the fat globules hardly contribute, but the pH is, of course, the same Changes caused by heat treatment are discussed in Section 10 1, those caused by concentrating in Section 17 2 (See also Section 4 5 )

## 11.4. REDOX POTENTIALS

Oxidation reduction reactions involve transfer of electrons between atoms or molecules Transfer of O or H, or both, also may occur Oxidation and reduction are, respectively, loss and gain of electrons If there is no flow of electrons into or out of the system, redox reactions are necessarily coupled, a half-cell oxidation reaction occurring simultaneously with a half cell reduction reaction

If a compound, Ox, is reduced reversibly to another, Red,

$$Ox + n\,e \leftrightharpoons Red \tag{11 8}$$

as, for example,

$$Fe^{3+} + e \leftrightharpoons Fe^{2+} \tag{11 9}$$

the potential of the system to transfer electrons can be measured by inserting into it an inert electrode such as gold or platinum Electrons can pass back and forth between the system and the metal The potential actually is measured by connecting this half cell via salt bridges through a potentiometer to another half cell of known potential All potentials are referred to a common standard, the H half cell (H electrode)

$$1/2\ H_2 \leftrightharpoons H^+ + e \tag{11 10}$$

whose potential is assigned a value of zero when an inert electrode dips into a solution of unit activity with respect to protons (pH = 0) in equilibrium with $H_2$ gas at a pressure of 1 atm The oxidation reduction or redox potential, $E_h$, is expressed in volts It is related to the concentrations of the oxidized and reduced forms of the compound as follows

$$E_h = E_o + \frac{RT}{nF} \ln\left(\frac{[Ox]}{[Red]}\right) \tag{11 11}$$

where $E_h$ = redox potential (V)

$E_o$ = standard redox potential (V); i.e., the potential when the reactant and product are both at unit activity

$n$ = number of electrons transferred per molecule

$R$ = gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$)

$T$ = absolute temperature

$F$ = Faraday constant (96.5 $kJ \cdot V^{-1} \cdot mol^{-1}$)

[Ox] and [Red] = activities of Ox and Red, respectively

At 25°C and one electron transfer the equation becomes

$$E_h = E_o + 0.059 \log ([Ox]/[Red]) \qquad (11.12)$$

According to the convention used by biochemists, $E_h$ becomes more positive as the ratio [Ox]/[Red] increases. $E_o$ is a characteristic of each oxidation-reduction system and is a measure of the relative ability of the system to accept or donate electrons. With dilute systems it is customary to use molar concentrations of the components rather than activities in the calculations.

In many systems of biological importance $H^+$ ions enter into the overall chemical reaction of the half cell. In such systems the $E_h$ will vary with pH as follows when one electron transfer is involved:

$$E_h = E_o' + 0.059 \log([Ox]/[Red]) - 0.059 \text{ pH} \qquad (11.13)$$

and also the standard potential $E_o$ thus depends on pH. (The coefficients are 0.059/2 when the reaction involves transfer of two electrons.) A system exhibits poising (analogous to buffering in proton equilibria) at $E_h$ values close to its $E_o$, hence at ratios of [Ox]/[Red] close to unity.

In Figure 11.6, $E_h$ of milk and $E_o$'s of several systems of importance pertinent to it are plotted against pH. Individual milk samples in equilibrium with air generally have $E_h$'s in the range of +0.25–+0.35 V at 25°C at their normal pH of 6.6–6.7. Not much information is available on the capacity of the oxidation-reduction systems in milk. One study reported that 1.6 mEq of $FeSO_4$ was required per liter to bring the $E_h$ at pH 6.6 down to +0.09 V. Another study of 58 fresh milk samples found 1.5 to 7.5 mEq per liter required to lower the $E_h$ (at 37°C) to −0.30–−0.35 V. Fresh raw whole milk will reduce 0.6–0.8 mmol of ferricyanide to ferrocyanide per liter at 50°C for 20 min.

Besides the redox potentials, which pertain to equilibrium conditions, the kinetics of the oxidation-reduction reactions are important. Some of the reactions are very slow, because of a high free energy of activation ($\Delta G^*$—see Section 10.2), which implies that it may take considerable time before equilibrium is reached. Accurate measurement of $E_h$ may thus be time consuming, certainly in milk, which has several overlapping systems some of which are sluggishly or even incompletely reversible. Back diffusion of $O_2$ in the sample during the measurement may complicate matters further.

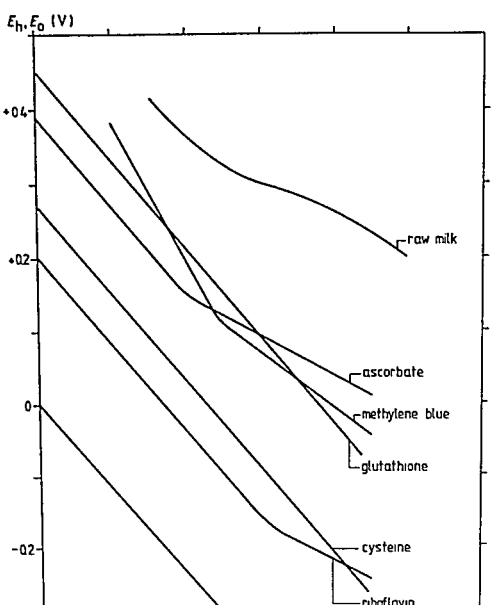



**Figure 11.6.** The redox potential ($E_h$) of milk and the standard potentials ($E_o$) of various systems in relation to pH After various sources

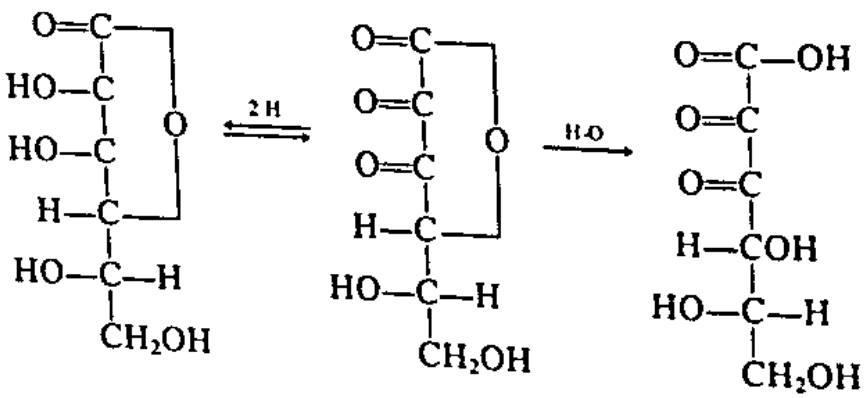
Meaningful data on the poising index (analogous to the buffer index) therefore cannot be obtained, though milk has considerable poising capacity

The principal systems that determine the $E_h$ of milk are $O_2$, ascorbate, and riboflavin In heated milk the thiol–disulfide system contributes also The lactate–pyruvate system is not reversible unless activated enzymatically and, in any event, is present in negligible concentration in fresh milk The aldehyde group of lactose is oxidizable to carboxyl at high pH, but this is not a reversible system contributing to $E_h$ at pH 6 6

Oxygen accepts four electrons and four protons per $O_2$ molecule in being reduced to two molecules of water. The $E_0$'s of these steps are positive, indicating strong tendency to accept electrons. As secreted, milk is almost oxygen-free, but after equilibration with air its $O_2$ content is about 0.3 m*M* (1.2 mEq·liter$^{-1}$). That $O_2$ is the major determinant of $E_h$ is demonstrated by:

1. The drop in $E_h$ to about $-0.15$ V when $N_2$ is bubbled through milk to sweep the $O_2$ out.
2. Reduction of methylene blue by anaerobically drawn milk and loss of this reducing power when such milk is exposed to air.
3. The drop in $E_h$ and the regain of the ability to reduce methylene blue when bacteria such as *S. lactis* or lactobacilli grow in milk. Of course, in this case the drop in pH, which tends to raise $E_h$, is involved as well.

The ascorbate content of fresh milk is about 0.25 mEq·liter$^{-1}$. As milk is drawn from the udder the ascorbate is all in the reduced form, but reversible oxidation to dehydroascorbate occurs at rates dependent on temperature and Cu and $O_2$ concentrations. The lactone ring of the dehydroascorbate is split irreversibly with the formation of 2,3-diketo-L-gulonate:




(11.14)

When milk is held at temperatures of 1–4°C (to inhibit the growth of microorganisms), reduced ascorbate gradually decreases over several days, and dehydroascorbate remains rather constant at about 0.05 mEq·liter$^{-1}$. Thus, the ratio of ascorbate to dehydroascorbate remains large until the system disappears. Ascorbate is preserved in milk by prevention of contamination with Cu and by deaeration. Some studies have found a significant inverse correlation between $E_h$ and content of reduced ascorbate in fresh milk (presumably equilibrated with air). In one such study raw milks from thirty individual cows examined within two hours of milking had $E_h$'s of 0.30–0.37 V and ascorbate contents of 0.15–0.30 mEq·liter$^{-1}$.

The concentration of free riboflavin (Figure 11.7) in milk is only about 4 $\mu M$. Its importance in oxidation-reduction phenomena resides in its role in

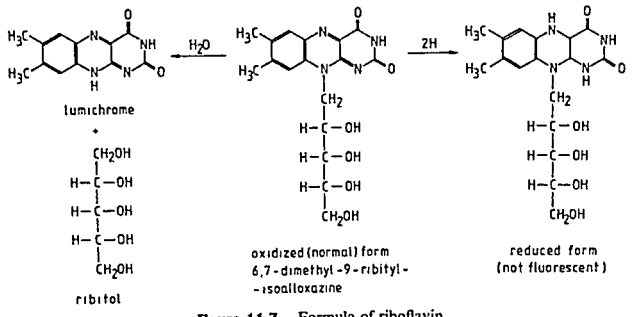
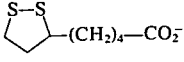



**Figure 11.7.** Formula of riboflavin.

photooxidation rather than in any significant contribution to $E_h$ or poising action. Milk contains only low concentrations of small molecular weight thiols. From rather scanty analyses it may be calculated that the concentration of lipoate (thioctate)



is on the order of 0.06 mEq·liter$^{-1}$. Only traces or no free cysteine and no glutathione have been reported. Thiols of the native proteins are not active in oxidation-reduction systems, but they are made so when the proteins are unfolded and uncoiled by heat denaturation. The concentration of such activated thiols in milk heated under UHT conditions (such as 150°C for 10 s) so as to avoid their oxidation may attain 0.18 mEq·liter$^{-1}$ (as determined by the capacity to reduce thiamin disulfide). These groups no doubt are mostly in the $\beta$-lactoglobulin, which has one thiol · mol$^{-1}$, equivalent to about 0.16 mEq·liter$^{-1}$ of milk. $E_o$'s of thiol compounds differ with structure as indicated by the difference between glutathione $HO_2C$-$CH_2$-NH-CO-CH($CH_2SH$)-NH-CO-$CH_2$-$CH_2$-CH(-$NH_2$)-$CO_2H$ and cysteine HS-$CH_2$-CH(-$NH_2$)-$CO_2H$ in Figure 11.6. $E_o$'s for the protein-bound thiols of milk proteins are not known, and the system may not be readily reversible.

## 11.5. REDOX REACTIONS AND PHOTOOXIDATION

Redox reactions (transfers of electrons) in milk systems are influenced by heat treatment, by concentrations of $O_2$ and metal ions such as $Cu^{2+}$, by exposure to light, and by oxido-reductases of milk and of microorganisms.

The redox potentials of metal ions may depend closely on their binding to various ligands, including some (metallo) proteins.

Heat treatment of milk activates protein thiols. If oxygen is present, heating accelerates the oxidation of thiols and the destruction of the ascorbate–dehydroascorbate system. Heating promotes Maillard reactions between lactose and proteins (Section 10.4) with the production of reductants of the enediol type. These various reactions differ in activation energy. (See Section 10.2.) Consequently, the overall balance of the redox system after heat treatment depends greatly on the time and temperature used. High-temperature short-time heating leaves more reduced ascorbate and activated thiols than do low-temperature short-time treatments. The rate of production of reductants by the Maillard reaction increases with concentration of reactants. Thus, more of these compounds result from heat treatments applied in concentrating and drying than from those used on unconcentrated milk. The total reducing capacity of milk for ferricyanide at pH 6.6 (20 min at 50°C) may be increased several-fold by concentrating and drying treatments but not all of the compounds produced form readily reversible redox systems. Thus, $E_h$ may remain high in the presence of high total reducing capacity. Table 11.3 gives some examples of the effects of heat treatments on reducing capacity and $E_h$. Some $H_2S$ is formed during heating, especially of cream, and it may bind $Cu^{2+}$ thereby decreasing its prooxidant capacity.

Molecular $O_2$ exists in the relatively unreactive triplet state $^3O_2$. This has two unpaired electrons in the $2p\pi$ orbital. Some of the activated states and reduction products of $O_2$ are much more powerful oxidants than triplet $O_2$.

The steps in reduction of $O_2$ may be formulated as follows:

$$O_2 + H^+ + e^- \longrightarrow \underset{\text{perhydroxyl radical}}{HO_2^\cdot} \rightleftarrows H^+ + \underset{\text{superoxide anion radical}}{O_2^{\cdot-}} \qquad (11.15)$$

The equilibrium of the second reaction is far to the left at the pH of milk. The perhydroxyl radical is further reduced according to

$$HO_2^\cdot + H^+ + e^- \rightarrow \underset{\text{hydrogen peroxide}}{H_2O_2} \rightarrow 2\,H^+ + \underset{\text{peroxide ion}}{O_2^{2-}} \qquad (11.16)$$

$$H_2O_2 + H^+ + e^- \rightarrow \underset{\text{hydroxyl radical}}{HO^\cdot} + H_2O \qquad (11.17)$$

**Table 11.3. Effect of Heating and Drying on Reducing Capacity and $E_h$ of Milk**

| Treatment | | | | Ascorbate (mEq liter $^{-1}$) | Ferri-cyanide[b] | Thiamin Disulfide[c] | $E_h$ (mV) |
|---|---|---|---|---|---|---|---|
| Temp (°C) | Time (min) | Atm | Dried[a] | | | | |
| | Raw Control | | | 0 13 | 0 65 | — | ~315 |
| 85 | 30 | air | − | 0 10 | 1 10 | 0 08 | 200 |
| 85 | 30 | air | + | 0 03 | 1 10 | 0 06 | 370 |
| 85 | 30 | $N_2$ | − | 0 12 | 0 85 | 0 13 | 0 |
| 96 | 30 | air | − | 0 04 | 0 90 | 0 10 | 350 |
| 96 | 30 | air | + | 0 04 | 1 10 | 0 06 | 360 |
| 96 | 1 3 | air | − | 0 09 | 0 80 | 0 13 | 300 |
| 96 | 1 3 | air | + | 0 07 | 1 15 | 0 10 | 345 |

[a] And reconstituted before analysis

[b] Reduction of ferricyanide in mEq liter$^{-1}$ at pH 6 6 during 20 min at 50°C

[c] Reduction of thiamin disulfide in mEq liter $^{-1}$ at pH 6 6 during 2 h at 25°C After H A Harland, R Jenness and S T Coulter, *J Dairy Sci* 35 (1952) 643

$$HO\cdot + H^+ + e^- \rightarrow H_2O \rightleftarrows H^+ + OH^- \qquad (11.18)$$

hydroxyl anion

Superoxide anion, $O_2^-$, can be formed in milk in various ways, including

$$Cu^{2+} + \text{ascorbate} + 2\,{}^3O_2 \rightarrow O_2^- + 2\,H^+ + \text{dehydroascorbate} \qquad (11.19)$$

$$\text{Reduced riboflavin} + 2\,{}^3O_2 \rightarrow 2\,O_2^- + \text{oxidized riboflavin} + 2\,H^+ \qquad (11.20)$$

$$\text{Reduced riboflavin} + 2\,{}^3O_2 \rightarrow 2\,O_2^- + \text{oxidized riboflavin} + 2\,H^+ \qquad (11.20)$$

$$\text{Substrate} + {}^3O_2 \xrightarrow[\text{oxidase}]{\text{xanthine}} O_2^- + \text{oxidized substrate} \qquad (11.21)$$

There is some evidence that superoxide anion or, more likely, perhydroxyl radical serves as an oxidant of unsaturated lipids, but current opinion is that the principal agent initiating the oxidation of lipids is singlet oxygen. (See also Section 5.6.)

Singlet oxygen, $^1O_2$ or $^1\Delta O_2$, is an activated species in which one of the oxygen atoms has two paired $2p\pi$ electrons, and the other has none in the $2p\pi$ orbital. Thus, it is highly electrophilic and avidly seeks to fill its vacant molecular orbital. It can be generated in several ways, of which the following can be pertinent to milk:

$$H_2O_2 + O_2^- \rightarrow OH^- + OH\cdot + {}^1O_2 \qquad (11.22)$$

$$OH\cdot + O_2^- \rightarrow OH^- + {}^1O_2 \qquad (11.23)$$

$$H_2O_2 + HO_2\cdot \rightarrow OH^- + H_2O + {}^1O_2 \qquad (11.24)$$

The significance of these reactions in producing singlet $O_2$ in milk has not been well established. If milk is illuminated, some $^1O_2$ is formed via the formation of triplet-excited riboflavin (Fl*):

$$\text{Riboflavin } h\nu \rightarrow Fl^* \qquad (11.25)$$

$$Fl^* + {}^3O_2 \rightarrow \text{riboflavin} + {}^1O_2 \qquad (11.26)$$

Some workers in the field consider that metal-catalyzed oxidation in milk proceeds by means of formation of superoxide anion by oxidation of ascorbate (Equation 11.19) and that the $O_2^-$ reacts with $H_2O_2$ (Equation 11.22) to form singlet $O_2$. Evidence for this idea is that compounds that trap or quench singlet oxygen greatly inhibit Cu-catalyzed oxidation of milk lipids. Addition of the enzyme superoxide dismutase likewise inhibits Cu-catalyzed oxidation, and it is considered that this enzyme causes dismutation of $O_2^-$ to $H_2O_2$

and triplet $O_2$ rather than singlet $O_2$ Incidentally, oxidation of ascorbate by chelated Cu, as in the enzyme ascorbate oxidase, occurs by a different mechanism and transfers four electrons and protons to $O_2$ to form water rather than $O_2^-$ or $H_2O_2$

The reactions described in Equations 11 20, 11 25, and 11 26 are involved in *photooxidation* Riboflavin absorbs visible light of wavelengths around 450 nm and is thereby excited to a triplet state (Equation 11 25) This excited-state riboflavin can oxidize a number of compounds, being itself thereby reduced The reduced riboflavin (Figure 11 7) can react with triplet $O_2$, as shown in Equation 11 20, to produce superoxide anion (or $H_2O_2$ at low pH) Compounds in milk that can be oxidized by excited riboflavin are ascorbate, methionine, cysteine, histidine, tyrosine, tryptophan, and orotate The proteins $\alpha$-lactalbumin, $\beta$-lactoglobulin, and serum albumin are also oxidizable in this way as judged by production of superoxide anion when they are illuminated with light from fluorescent lamps at pH 6 7 in the presence of riboflavin at a concentration of 3 2 $\mu M$ (similar to its concentration in milk) The production of superoxide anion in these reactions was enhanced if the proteins had previously been heated at 63°C for 30 min, suggesting that the amino acid residues are made more available to oxidation as the protein unfolds and uncoils Some aggregation and degradation (cleavage of peptide bonds) occurs A very striking result of these reactions in the production of methional ($CH_3$-S-$CH_2$ $CH_2$-CHO), which seems to be largely responsible for the off-flavor in milk that has been exposed to light

Another photooxidative pathway involves direct reaction of excited riboflavin with $^3O_2$ to form singlet oxygen $^1O_2$ (equation 11 26) This is probably not the main fate of excited flavin, but it may provide the main pathway in producing singlet $O_2$ in milk (equation 11 27) It should be noted also that riboflavin is degraded by the action of light, the ribityl group is cleaved from the molecule with the formation of lumichrome (6,7 dimethylalloxazine—see Figure 11 7) This process proceeds with first-order kinetics, the rate being about $10^{-5}$ $s^{-1}$ (50 ml samples of whole or skim milk exposed to ~ 2500 lux of fluorescent light)

Photooxidation reactions in milk depend on intensity and wavelength of the light, but few quantitative studies are available Riboflavin absorbs nearly 470 nm, so short wavelength light is more active, direct sunlight and fluorescent lamps (TL lamps) have stronger effects than incandescent lamps Putting a 1-liter glass bottle of milk for 10 min in direct sunlight usually suffices to produce a distinct off flavor, to be sure, the flavor becomes noticeable only after some hours as it originates from reaction products formed after photooxidation (See above ) Most photochemical reactions are independent of temperature because light quanta rather than the kinetic energy of the molecules provide the activation energy Ensuing reactions nevertheless may depend on temperature

The *oxido-reductase enzymes* in milk ordinarily are only little involved in redox reactions occurring therein Ten such enzymes are listed in the

appendix (Table A.10) as occurring in milk. Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) are NAD-dependent enzymes (NAD = nicotinamide-adenine dinucleotide). No doubt, LDHs of bacteria are involved in the synthesis of lactate from lactose (Section 3.3.1) as milk sours, but there is no evidence of the further participation of either milk or bacterial LDH or MDH in redox reactions. Xanthine oxidase is, of course, prominent in bovine milk. If furnished with substrate (xanthine, hypoxanthine, aldehydes) it would certainly function and produce $H_2O_2$ or $O_2^-$. There seem to be little if any endogenous substrates in milk upon which xanthine oxidase can work. The activities of lactoperoxidase, catalase, and superoxide dismutase all involve $H_2O_2$ or $O_2^-$. Thus, they might be coupled to the activity of xanthine oxidase. Sulfhydryl oxidase may catalyze oxidation of thiols to disulfides in milk. As mentioned in Section 7.3.1, its use to oxidize thiols in UHT milk to reduce cooked flavor has been proposed.

Some of the enzymes are metalloproteins (e.g., xanthine oxidase, lactoperoxidase), and they may become prooxidants upon denaturation as caused by heating; this is not an enzyme activity.

## 11.6. MILK AS A SUBSTRATE FOR BACTERIA

Numerous microorganisms, predominantly bacteria, can proliferate in milk, thus changing its properties. Generally, significant changes in composition of the milk are not detected until the bacterial count has reached, say, $10^6$ $ml^{-1}$ or more, though this depends greatly on the type of bacteria present and on the duration of the experiment. Bacterial growth and action in milk may be either undesired (spoilage, growth of pathogens, formation of toxins) or deliberately induced, as in the manufacture of fermented products (e.g., cheese, yogurt, sour cream, kefir). This book is not the place to discuss these topics, and we will restrict ourselves to the influence of the properties of milk and milk products on growth and metabolic action of bacteria. This is to some extent an oversimplification, because milk products are ecosystems. In other words, the interaction between bacteria and environment determines what will happen; bacterial action affects the environment, and the latter determines which bacteria can proliferate. The environment includes the properties of the substrate (i.e., milk or a derivative) and outside conditions, of which temperature is by far the most important variable. The bacterial population can vary greatly. Raw milk left in contact with the outside world is essentially an open ecosystem; almost any bacterium can be present, and the properties of the milk and the temperature largely determine which bacteria will outmatch the others. Many milk products are closed or controlled ecosystems, and the microbial changes occurring depend greatly on the particular contamination with bacteria that has occurred. Not only species and numbers of bacteria are important, but also their physio-

logical condition (e.g., stage of growth, adaptation to milk) and the possible presence of bacteriophages. The effect of the environment is usually different for growth, fermentation, and, in the case of certain species, sporulation. Generally, conditions permitting growth are more restricted than those for fermentation. For instance, several lactic acid bacteria do not grow to any extent near 5°C, but, other conditions being favorable, they may go on producing lactic acid from lactose. Conditions inducing sporulation, whence possible growth and fermentation of the ensuing bacteria, are usually rather restricted.

Milk contains such a wide range of nutrients, including all of the vitamins, that numerous species of bacteria find sufficient raw material for fermentation and growth. The result is well known: Raw milk spoils rapidly at room temperature. But because the bacteria that can grow in milk may have very different properties, we should be cautious in applying general rules. For some bacteria, lactose is not a suitable energy source. Others rely on free amino acids as an N source, and fresh milk contains only tiny amounts of amino acids. Consequently, such bacteria often start to grow after other bacteria have hydrolyzed proteins, thus providing suitable nutrients. Another such example is the production of $CO_2$ by some lactic streptococci, which stimulates growth of some lactobacilli. (On the other hand, some Gram-negative bacteria are inhibited by $CO_2$.) Some bacteria need specific trace components for growth or fermentation that are missing or present in insufficient concentrations. An example is Mn, needed by *Leuconostoc cremoris* for the fermentation of citric acid, which yields aromatic compounds that may be desirable; Mn content of milk often varies with season. (See Figure 9.8.)

Some conditions in milk may be unfavorable for growth of some bacteria. A possible lack of nutrients has been mentioned already. Water activity and ionic strength of milk are never limiting, and pH is so only for a few organisms. But redox potential and $O_2$ pressure are mostly such that strictly anaerobic bacteria cannot grow. The growth of aerobic bacteria also depends on the location; in a cream layer $O_2$ pressure may be much higher than near the bottom of a deep vessel of milk. Bacterial action, if at room temperature, usually lowers both $O_2$ level and pH. For very few microorganisms are conditions in milk so unfavorable as to kill them.

Milk contains natural *inhibitors*. Some bacteria do not grow in milk despite the presence of sufficient nutrients and suitable conditions. A mere delay (or lag phase) in growth after adding the bacteria to the milk is not proof of inhibition; the bacteria may not be adapted to milk (i.e., they have to change their enzyme system before they can use the nutrients available).

An important class of inhibitors is the immunoglobulins (Section 6.4.4), which are antibodies against specific antigens, often bacteria. They are thus specific for the species and strains of bacteria encountered by the cow, and other bacteria are not inhibited. Mixed milk may contain immunoglobulins active against a wide variety of bacteria, but the concentration is usually

low. Though examples of inhibition by IgG and IgA (probably in cooperation with "complement") in milk are known, the agglutinative action of IgM is most conspicuous. There is an agglutinin acting against strains of *S. pyogenes*, another against strains of *S. lactis* and *S. cremoris*.

Agglutination means that bacteria meet each other owing to their Brownian motion and are kept together in (large) floccules by the sticking action of the agglutinin. The floccules can become so large that they sediment, removing the bacteria from most of the milk. Bacteria also can become attached by agglutinins to fat globules if the temperature is low. The cold agglutination and rapid creaming of the fat globules (Section 14.3) then cause rapid removal of bacteria from most of the milk. The organisms are not killed but restricted in growth, because of the depletion of nutrients and the accumulation of inhibiting metabolites in the sedimented layer of floccules. Those concentrated in the cream layer also may be inhibited by the higher $O_2$ pressure. If agglutination is hindered mechanically, for instance when a milk is renneted directly after adding a starter (the bacteria now become enclosed in the meshes of the paracaseinate network), inhibition is insignificant. The content of agglutinins in milk is highly variable; usually, colostrum has relatively high concentrations.

Some nonspecific bacterial inhibitors are lysozyme and lactoferrin, but their concentrations in cow's milk are so low that they have little effect; human milk contains much more. Lysozyme is an enzyme (EC 3.2.1.17) that hydrolyzes polysaccharides of the bacterial cell wall, particularly splitting off N-acetyl muramic acid; this causes lysis of the bacteria. Lactferrin binds Fe, thus reducing the activity of $Fe^{2+}$ ions, which are needed by several bacteria.

The most important nonspecific inhibitor of milk is the peroxidase-thiocyanate-$H_2O_2$ system, which is also quite active in saliva. The milk enzyme lactoperoxidase (EC 1.11.1.7) as such does not cause inhibition, but it catalyses the oxidation of $SCN^-$ by $H_2O_2$, and one of the intermediates ($OSCN^-$?) is a powerful bacteria killer. Milk contains far more than sufficient peroxidase, up to 0.4 $\mu M$. The $SCN^-$ content is variable (it depends on feed, as it is mainly derived from glucosides in species of *Brassica* and *Raphanus*) and mostly ranges from 0.02–0.25 m$M$. If the concentration is indeed 0.25 m$M$, $SCN^-$ is, in conjunction with peroxidase, active against many bacteria that have no catalase and thus produce $H_2O_2$ (e.g., all lactic acid bacteria, although some of them have enzyme systems that metabolize $H_2O_2$ so that they are not inhibited or are less so). Fresh milk contains no $H_2O_2$, but if a little is added, say 0.25 m$M$ (i.e., far too little to be active as such against bacteria or to cause oxidative defects), the system also inhibits catalase-positive organisms, like most Gram-negative bacteria. If sufficient $SCN^-$ is also present (either naturally or added), an effective preservative results, and even in a heavily contaminated milk bacterial growth may be prevented for, say, 24 h at 15°C. Addition of some glucose oxidase (EC 1.1.3.4/5) also

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induces formation of $H_2O_2$, and it may be that the natural milk enzyme xanthine oxidase (EC 1 2 3 2) can do the same under some conditions The natural content of catalase (EC 1 11 1 6) in milk does not interfere with the system, even if it is high (mostly because of a high somatic cell count) Nevertheless, the inhibitory effect in milk is quite variable, largely because of the variation in thiocyanate content

Inhibitors may enter the milk by contamination This is undesirable, not only because fermentation may be impaired but also because these substances may be detrimetal to the consumers' health Antibiotics like penicillin may be present because they have been infused into the udder to control mastitis, and they can be found in the milk up until about three days after the infusion Particularly, some lactic acid bacteria are sensitive to these antibiotics Disinfectants used to treat milking or processing equipment may contaminate the milk easily and then inhibit or even kill bacteria In some countries $H_2O_2$ may be added to raw milk as a preservative (10–15 m*M*), it is removed by addition of catalase before processing of the milk

*Treatment of milk* may profoundly alter its suitability as a substrate for bacteria The most important is heat treatment (Chapter 10), which kills bacteria and may activate sporulation but also alters the milk Inhibitors are inactivated, this pertains to the immunoglobulins (if heated for, say, 20 s at 76°C) and to the lactoperoxidase system (e g , 20 s at 85°C) Consequently, pasteurization may stimulate considerably growth of bacteria (which have entered the milk afterwards), the higher the heating intensity the more, up until around 20 s at 85°C Still more intense heating may lead to formation of stimulants, such as formic acid for certain lactobacilli

Homogenization appears to inactivate the agglutinins, but it usually is applied to milk that has been heated to such an extent that the agglutinins are inactivated anyhow

Fermentation by lactic acid bacteria causes the formation of lactic acid, and this is an effective inhibitor for many bacteria if it is undissociated Its p*K* is about 3 95, which implies that the inhibition is stronger for a lower pH Hardly any bacteria can grow in milk brought to pH of less than 4 5 by lactic acid, but some yeasts and molds can Bacteria also can produce other inhibiting substances, such as acetic acid, and antibiotics Some strains of *S lactis* produce the powerful antibiotic nisin

Fermentation implies a drastic change in composition Other such changes also may be effective in inhibiting bacteria Conditions can be made strictly anaerobic The water activity can be lowered to such an extent that no bacteria can grow (Section 17 1), as in milk powder (by removing water) and in sweetened condensed milk (by adding sugar) The salt added to cheese has a similar effect and also increases ionic strength Often, a combination of lack of suitable nutrients, unfavorable conditions, and inhibiting agents prevents growth, this is notably true in many types of cheese (no sugar, low redox potential, not too high temperature, high salt, low pH, lactic acid)

## *Suggested Literature*

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# 12

# Colloidal and Surface Phenomena

A glance back at Table 1.2 shows that colloidal aspects must be important for milk and its derivatives. Milk has many structural elements of colloidal dimensions. The particles vary widely in size and composition, and some of them have a complicated structure; all carry a surface charge. They have large surface areas that interact with the many surface-active components of milk.

The fat globules and the casein micelles are particularly subject to many alterations. Some of these changes are essential in such dairy manufacturing processes as churning and renneting. Others affect the physical properties and stability of milk and milk products. The interactive forces between the particles often play a decisive role in these processes.

This chapter serves two purposes. It is mainly an introduction to Chapters 13–16 and parts of Chapter 17, for the benefit of readers not entirely familiar with the underlying principles of the physical chemistry of surfaces and colloids. It also relates these principles to milk. Consequently, some general colloidal and surface aspects of milk and its derivatives are treated here rather than in the more specialized chapters following.

## 12.1. SURFACE CHEMISTRY

### 12.1.1. Surface Tension

Free energy is stored in the interface between two phases. A phase is a domain bounded by a closed surface in which the intensive parameters (such as composition, temperature, pressure, and refractive index) are constant,

while at least some of these parameters change abruptly at the surface. Such changes occur in fact over distances of a few times molecular size, so the condition of abruptness implies that the domain must have a size many times that of the constituting molecules. The principal phases of milk, then, are serum and liquid fat; if present, air bubbles and the crystals of fat, ice, and lactose constitute phases. Casein micelles, though colloidal particles, cannot be considered as a distinct phase; they are not sufficiently homogeneous and have no sharp boundaries. Consequently, milk plasma behaves in many respects like a single phase. Also, the fat globule membrane is not a phase; its dimension in the radial direction is far too small.

Increase of interfacial area means increase of interfacial free energy. Hence, an interface resists enlargement, which implies that a force is acting in the direction of the interface. It can be expressed as a two-dimensional tension (force per unit length: $N \cdot m^{-1}$) called the interfacial tension, $\gamma$. Numerically, it equals the interfacial free energy (expressed in energy per unit area: $J \cdot m^{-2} = N \cdot m^{-1}$). When the interface is between a solid or liquid and a gas, particularly air, it is common to call it a surface and to speak of surface tension.

The interfacial tension depends on the composition of the two phases and on substances adsorbed. Some values are given in Table 12.1. $\gamma$ decreases somewhat with increasing temperature.

### 12.1.2. Adsorption

Substances dissolved in one or both of the phases may accumulate into or adsorb onto the interface. If both phases are fluids, the interface has no specific adsorption sites, and a solute can be adsorbed until the interface is packed with a monomolecular layer. The solute is adsorbed if it lowers the interfacial tension. This is expressed by Gibbs's equation:

$$d\gamma = -RT\,\Gamma\, d \ln a \qquad (12.1)$$

where $\Gamma$ is the surface excess or adsorbed amount (here in moles per unit area) and $a$ is the thermodynamic activity of the solute in the bulk phase. Because the activity often is unknown, we usually take the concentration $c$ of the solute instead ($c$ as mole fraction), which may be fairly correct for low concentrations; $c$ should be taken in the bulk phase, not in the whole system.

Equation 12.1 implies that, for a solute that lowers $\gamma$, surface excess increases and $\gamma$ decreases with increasing concentration. Different solutes are adsorbed to different extents at the same concentration, and the same $\gamma$ may be reached with very different solute concentrations. (See also Na laurate and Na stearate in Table 12.1.) Some substances even tend to stay away from the interface (negative adsorption) so that $\gamma$ increases with increasing concentration. This is true for most salts in aqueous solution.

We will further consider air–water (AW) and oil–water (OW) interfaces. Water is, by definition, hydrophilic; the other fluid (air or oil) is hydrophobic.

**Table 12.1. Some Examples of Interfacial Tensions ($\gamma$)**

| Interface between Phases | $\gamma$ (mN m$^{-1}$) |
|---|---|
| Water–air | 72 |
| 22 m*M* Na laurate in water–air | 43 |
| 0 3 m*M* stearate in water–air | 43 |
| *n* octane–air | 22 |
| Water–*n* octane | 51 |
| Milk plasma–air | 48 |
| Sweet-cream buttermilk–air | 40 |
| Liquid milk fat–air | 34 |
| Liquid milk fat–water | 20 |
| Liquid milk fat–milk plasma | 15 |
| Liquid milk fat–protein solutions | 10–15 |
| Milk fat globule–milk plasma | 2[a] |
| Liquid fat–fat crystal ($\alpha$ modification) | 10 |

*Note* Approximate values at 20–40°C
[a]Measured values range from 1 –2 5 mN m$^{-1}$

Adsorbing substances, often called surfactants, are usually amphiphilic molecules Examples are polar lipids (phospholipids, monoglycerides), fatty acids or soaps, and synthetic surfactants like polyoxyethylene sorbitan monolaurate (See Figure 12 1 ) They tend to lower the surface tension of water $\gamma_{AW}$, to roughly the same extent as they lower $\gamma_{OW}$, which implies that the latter can become very low, even less than 1 mN m$^{-1}$ for some surfactants

Certain macromolecules also may adsorb onto an interface, but Equation 12 1 no longer may apply It is valid for a dynamic equilibrium, such as exhibited by small-molecule surfactants, they move in and out of the interface with an average residence time much shorter than the time scale of an experiment Many macromolecules, however, are adsorbed more or less irreversibly, their residence time may be much longer than the experiment

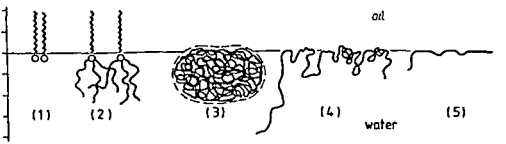



**Figure 12.1.** Adsorption of substances onto an oil–water interface (1) ' soap (2) tween (3) (4) and (5) proteins in various states of unfolding Highly schematic On the left is a scale of nanometers Often polymer chains will extend further into one of the phases

lasts, and equilibrium is not attained. Moreover, they tend to rearrange their conformation on adsorption. Given time and space (which implies that few macromolecules are available per unit interfacial area), they may completely unfold and become stretched. More often, various segments along the chain will be adsorbed, while loops and tails dangle into one of the phases. (See Figure 12.1.)

Proteins, having both hydrophilic and hydrophobic parts, are surface-active macromolecules and are readily adsorbed onto OW and AW interfaces. Globular proteins, such as those in milk serum, may unfold and in some cases even be denatured on adsorption. The surface excess or protein load depends on the ratio of protein available to surface area. It may range from 0.5–3.0 $mg \cdot m^{-2}$. Usually, protein is abundant and loads of 2.5 $mg \cdot m^{-2}$ or more are obtained. (See also Fig. 17.12).

If different surfactants are present, the one giving the lowest $\gamma$ at the prevailing bulk concentration displaces the others until the condition of equation 12.1 is fulfilled for each. (Actually, thermodynamics of adsorption is somewhat more complicated.) Most proteins do not reduce $\gamma$ greatly (e.g., $\gamma_{ow}$ = 10–15 $mN \cdot m^{-1}$), and small-molecule surfactants may replace them. Thus, polyoxyethylene sorbitan monolaurate, at a concentration in the bulk phase (after adsorption) of only 0.01% (w/w), will displace protein nearly completely from an interface. Monoglycerides and soaps have similar but smaller effects at the same concentration. In normal milk the activities of small-molecule surfactants are low; they increase as a result of lipolysis, which yields free fatty acids and a small amount of monoglycerides. Consequently, lipolysis may lower the surface tension of milk, by some 5 $mN \cdot m^{-1}$ at most.

The kinetics of adsorption need to be considered also. If we assume adsorption of globular proteins to $\Gamma$ = 3 $mg \cdot m^{-2}$ and a bulk concentration in the solution (milk serum) of about $6 \times 10^6$ $mg \cdot m^{-3}$, the protein needed to cover the interface can be provided by a layer of liquid 0.5 $\mu$m thick. Diffusion theory indicates that a diffusion time $t_{0.5}$ for a change of 50% in concentration over a distance $l$ is given by $l^2 = Dt_{0.5}$. Taking for $l$ the above-mentioned 0.5 $\mu$m and for the diffusion coefficient of globular proteins $D$ = $5 \times 10^{-11}$ $m^2 \cdot s^{-1}$, then $t_{0.5}$ = 5 ms. Consequently, diffusion to the interface will be complete well within 0.1 s. This implies that "uncovered" interfaces, whether AW or OW, do not exist in fluid milk products except for a very short time. Particularly, such a thing as "free fat" is clearly impossible.

Surfactants present in very low concentrations need a long time to diffuse to the interface, and it usually takes minutes or even hours before a constant surface tension is reached. If a mixture of proteins is present, they all tend to be adsorbed in proportion to their rate of arrival at the interface. Proteins are adsorbed strongly and replace each other in the adsorbed layer only sluggishly if at all. Consequently, interfacial tension and composition of the adsorbed layer may depend much on the history of the interface and may keep changing for a long time.

Proteins also may bind many surfactants. This is one of the reasons why the activity of a surfactant in milk is often rather low. A well-known example is binding of free fatty acids by serum albumin. Sodium lauryl sulfate and some other surfactants bind strongly to proteins and even denature them. Consequently, the changes found in properties of milk products when adding surfactants need be caused not only by adsorption at phase boundaries; changes in protein properties (conformation, charge) may occur too.

### 12.1.3. Curved Interfaces

The pressure at the concave side of a curved interface is higher than at the convex side. The difference is called the Laplace pressure, which, for the simple case of a spherical surface of radius $r$, is given by

$$\Delta p = \frac{2\gamma}{r} \tag{12.2}$$

This implies, for instance, that a milk fat globule with a diameter of 1 $\mu$m has an overpressure of about 10 kPa ($\approx$ 0.1 atm). The globule resists deformation because deformation produces a local increase in curvature (decrease in $r$), hence an increase in $\Delta p$.

An air bubble in skim milk of 0.1 mm diameter would give a $\Delta p$ of some 2 kPa (i.e., 2% of atmospheric pressure). This means that the solubility of the air in the bubble is higher than that of the air above the liquid (where $r = \infty$). This is generally true also for nongaseous components in a particle. A more general form of Equation 12.2 is the Kelvin equation, which relates the solubility $P_r$ of a particle of radius $r$ to that of a very large particle $P_\infty$:

$$RT \ln \frac{P_r}{P_\infty} = \frac{2\gamma M}{\rho r} \tag{12.3}$$

where $M$ is molar mass and $\rho$ is mass density of the substance in the particles. As a result of the difference in solubility, large particles tend to grow at the expense of smaller ones, and the latter disappear; this is called Ostwald ripening. Generally, radii should be below 0.1 $\mu$m to exhibit much of this effect for solids or liquids, or below 100 $\mu$m for gases (which have a much lower value of $M/\rho$). An additional prerequisite for Ostwald ripening is that the substance in the particles be at least slightly soluble in the surrounding medium. Consequently, small fat globules in milk do not dissolve, but air bubbles do so rapidly. Also, fat crystals in liquid fat and lactose crystals in condensed milk exhibit Ostwald ripening.

### 12.1.4. Contact Angle

Where three phases meet, the angles that the interfaces make with each other at the boundary line are determined by the three interfacial tensions.

Examples are depicted in Figure 12.2. If one phase is a solid, the situation is determined by one contact angle and the convention is to measure it in the densest fluid phase. For the case of Figure 12.2(a) Young's equation states

$$\cos\Theta = \frac{\gamma_{AS} - \gamma_{WS}}{\gamma_{AW}} \tag{12.4}$$

If, however, $(\gamma_{AW} + \gamma_{WS}) < \gamma_{AS}$, the equation would give $\cos\Theta > 1$, which is clearly impossible. In this case the liquid (W) spontaneously spreads over the solid, because that gives the lowest total interfacial free energy. For the case of Figure 12.2(b), we have spreading of oil over the water surface if $(\gamma_{AO} + \gamma_{OW}) < \gamma_{AW}$.

The contact angle depends on the adsorption of surfactants because the interfacial tensions do. This also may determine whether spreading occurs. For instance, pure triglyceride oils do not spread on water, but natural fats spread because small quantities of surfactants lower $\gamma_{OW}$. Milk fat does not spread on a (skim) milk surface; the adsorbing proteins lower $\gamma_{AW}$ to such a value that $(\gamma_{AO} + \gamma_{OW}) > \gamma_{AW}$. (See Table 12.1.) On the contrary, milk fat as globules or somewhat larger amounts of liquid fat form lenses on a (skim) milk surface; the contact angle in the oil phase is low, generally below 20°. A prerequisite for lens formation is that the globules reach the surface; usually they do not because of repulsion. (See Section 12.2.) Milk fat will spread on a freshly made milk surface before the latter has acquired an adsorbed protein layer. Initial spreading rate increases with the size of the fat droplet reaching the surface; for milk fat globules it will be on the order of 1 mm·s$^{-1}$ or less. Adsorption takes less than 0.1 s (see above), so a spreading radius of some 10 $\mu$m may be expected.

If, in the case of Figure 12.2(a), $(\gamma_{AW} + \gamma_{AS}) < \gamma_{WS}$, this would give $\cos\Theta < -1$ or $\Theta > 180°$, which means that the liquid does not wet the solid. Similar reasoning can be applied to the case of Figure 12.2(c), only if the $\gamma$'s are such that there is a finite contact angle will the solid particle adsorb onto the liquid interface. If an adsorbed particle is removed from the interface, the interfacial free energy increases. For instance the energy needed to remove a spherical particle of 20 nm diameter with a contact angle of 30° from an OW interface would be on the order of twenty-five times its average

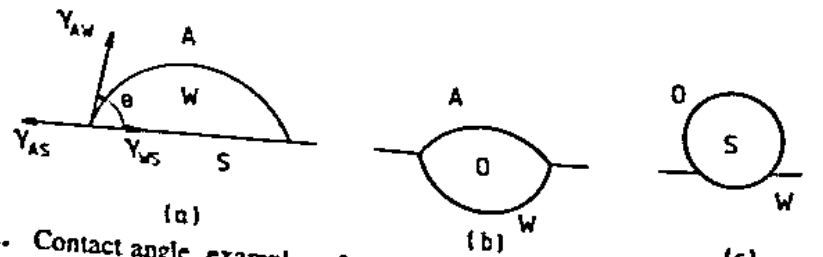



**Figure 12.2.** Contact angle examples of three-phase systems A = air, O = oil, S = solid, W = water.

kinetic energy, this implies that the adsorption is virtually irreversible More energy is needed for larger particles (proportional to diameter squared) and a contact angle closer to 90°

Fat crystals adsorb onto an OW interface and also onto the milk–fat milk–serum interface, but with a large contact angle (at least 150° as measured in the aqueous phase) They are, however, often prevented from reaching the interface by a repulsion barrier In butter, fat crystals help to stabilize the aqueous droplets against coalescence by being adsorbed onto them, sticking out into the oil phase, and thereby preventing contact between the droplets

Casein micelles can adsorb onto milk–fat–water and air–water interfaces However, they cannot be considered solid particles, and the reasoning given above does not apply, for instance, there is not a fixed contact angle Nevertheless, their adsorption lowers the total free energy if the interface is yet uncovered by other surfactants When they are adsorbed onto a clean OW interface, they also spread This is neither a spreading of a liquid (like oil over water) nor like the unfolding of a macromolecule It appears that the casein submicelles remain intact and form a layer at the interface Again, spreading occurs only as long as the interfacial tension has not been lowered too much by adsorption of other surfactants

### 12.1.5 Surface Tension Gradients

When surfactant molecules are not distributed evenly over an interface, surface tension gradients occur These are smoothed out rapidly because the surfactant molecules move until they are evenly distributed We may also say that the surface (interface) moves from regions with low $\gamma$ to those of high $\gamma$ The movement drags liquid near the interface with it This is called the Marangoni effect The streaming can be vigorous For an air–liquid surface, the stress $d\gamma/dz$ (where $z$ is the distance in the plane of the surface) must equal the shearing stress $\eta G_o$ (where $\eta$ is liquid viscosity and $G_o$ is the shear rate in the liquid at the interface) For instance, a $\Delta\gamma$ of 1 mN m$^{-1}$ over 10 $\mu$m distance causes for water a local shear rate of $10^5$ s$^{-1}$ Consequently, the effect may be large In practice, however, diffusion of surfactants to the interface may diminish greatly the development of surface tension gradients

Conversely, streaming of a liquid along an interface that contains surfactant causes a $\gamma$ gradient to develop In this way a liquid interface, like a solid interface, can withstand a tangential stress A completely clean OW interface cannot bear such a stress, if one liquid flows, the other must flow equally fast at the interface But even small amounts of surfactant prevent this effect, the interface acts more or less as a solid surface except for its deformability perpendicular to its plane For small droplets, the latter effect is counteracted by the Laplace pressure Consequently, milk fat globules act under most conditions as if they were solid particles

*Summarizing*, we may state that the adsorption of surfactants onto an interface has three main effects:

1. It lowers $\gamma$, hence Laplace pressure.
2. It permits development of $\gamma$ gradients.
3. It may alter the interaction forces acting between two approaching interfaces.

The last aspect is discussed in the next section.

## 12.2. COLLOIDAL STABILITY

Particles larger than most molecules but definitely too small to be visible are the subject of colloid science. The size range is approximately 1 to $10^5$ nm. Usually, reversible or lyophilic colloids are distinguished from irreversible or lyophobic colloids. When the continuous phase is aqueous, lyophilic and lyophobic mean hydrophilic and hydrophobic, respectively. Reversible colloids are thermodynamically stable; they are either macromolecules (such as proteins) or association colloids (micelles). Irreversible colloids constitute a true phase; in aqueous environment, this applies, for instance, to oil-in-water emulsions and to foams. Intermediates between the types mentioned may occur; casein micelles may be considered as such. In milk products fat globules are hydrophobic colloids; air bubbles and crystals of fat and lactose, if present, may also be so considered. Hydrophobic colloids are thermodynamically unstable:

1. They are subject to Ostwald ripening, particularly when very small. (See Section 12.1.3.)
2. They sediment or cream unless they are so small (say, $< 1$ $\mu$m) that Brownian motion prevails.
3. They tend to flocculate, particularly when not very small, though a repulsion barrier may postpone the flocculation for a very long time.
4. If they are fluid (fat globules at not too low temperature, air bubbles), they may coalesce with each other if the thin film of continuous phase between closely approaching globules or bubbles is ruptured somehow.

Two colloidal particles of equal composition always attract each other because of Van der Waals forces. If the attraction energy is on the order of $kT$ or smaller, Brownian motion suffices to keep the particles apart; $kT$ is the average collision energy between two molecules or particles ($k$ is the Boltzman constant, $1.4 \times 10^{-23}$ J·K$^{-1}$). But if the attraction is much stronger, there must be an energy barrier caused by some repulsion force to prevent

persisting contact between the particles. Repulsion is briefly discussed in Sections 12.2.3 and 12.2.4.

### 12.2.1. Hydrophilic Colloids

First, we will consider macromolecules, particularly polysaccharides and proteins. These are often thermodynamically stable in water (i.e., the free energy of the mixture is lower than that of the dry macromolecules plus that of water). Such reversible colloids are not fundamentally different from true solutions, though there may be additional factors leading to their stability, the main one being that the macromolecule can assume numerous different conformations in solution, thus increasing its conformational entropy. Conformation is also affected by the following factors:

1. Hydration (i.e., affinity between the macromolecule and water molecules) causes repulsion between different parts of the macromolecule as soon as the separation distance is on the order of a few water molecules.
2. Electrostatic repulsion occurs between like charges on a macromolecule.

Hence, pH may have a large effect; the further it is from the isoelectric point, the more extended the conformation. Near the isoelectric pH, attraction between opposite charges is likely, leading to internal salt bridges. These and other bonds between different parts of a macromolecule (e.g., hydrophobic interaction) cause a more compact conformation. A more compact conformation generally goes along with a lower solubility, and factors causing the macromolecule to extend also cause it to be more soluble. The effect of ionic strength is discussed in Section 12.2.2; aspects of protein solubility and conformation are presented in Section 6.1.

Most amphiphilic molecules (i.e., molecules with a hydrophobic and a hydrophilic part) are not very soluble in water or in apolar solvents. In both types of solvent they tend to associate into micelles, usually containing between ten and one hundred molecules, and in this way appreciable quantities apparently dissolve. Dissociated fatty acids (soaps) and phospholipids form roughly spherical micelles in water; the inside of such a micelle is hydrophobic, the outside hydrophilic. Such micelles are in equilibrium with single dissolved molecules and are thermodynamically stable. There is a critical micelle concentration (CMC) below which no micelles are formed and above which the activity of the solute hardly increases; almost all molecules added now go into micelles. For molecules with a long aliphatic tail, the CMC is low. Examples are Na-caprylate, 0.3600 *M;* Na-laurate, 0.0230 *M;* Na-stearate, 0.0004 *M*.

Amphiphilic molecules also adsorb onto an AW or OW interface. (See Section 12.1.2.) Usually, the bulk concentration at which they give a fully

packed monolayer at the interface equals the CMC. They also may be bound by other molecules, particularly proteins. Lipoprotein particles may be considered association colloids of mixed composition.

Monoglycerides and phospholipids present in oil also form micelles. In these the hydrophilic part is in the center; some water usually is needed to form these micelles.

### 12.2.2. The Electrical Double Layer

To understand the stability of lyophobic colloids, we need to consider attraction and repulsion in more detail. This also may be useful with respect to casein micelles. Adsorption of ions or ionization of groups on the surface of a particle gives it an electric charge. The surface has an electrostatic potential, $\psi_o$. This causes a repulsive force between particles with a $\psi_o$ of the same sign; the force increases with the absolute value of $\psi_o$. The repulsion, in turn, may prevent a close approach between the particles. The charge usually depends on pH, and so do $\psi_o$ and the repulsive forces.

The aqueous phase surrounding the particles always contains ions, and those of a charge opposite to that of the particle (the counterions) accumulate near its surface, thereby screening the charge. The closer to the surface, the higher the concentration of counterions. The layer with an excess of counterions is called the electrical or diffuse double layer. It sometimes is assumed that this layer is part of the particle and that the water molecules in the layer move with the particle. This is not true because ions and molecules in the double layer move freely; only the concentration of counterions is increased locally.

The electric potential of the particles diminishes with the distance $l$ from its surface. The relation is

$$\psi \approx \psi_o \, e^{-\kappa l} \tag{12.5}$$

$\kappa$ is the Debye–Hückel parameter. Its reciprocal is called the thickness of the double layer; it is the distance over which $\psi$ drops to $1/e$ equals 0.37 times $\psi_o$. It depends on the ionic strength of the solution. In water at room temperature (relative dielectric constant 78), it is given by

$$1/\kappa \approx 0.3/\sqrt{I} = 0.42 \left( \sum c_i z_i^2 \right)^{-1/2} \tag{12.6}$$

where $c$ and $z$ are molar concentration and valency of the ionic species present; $1/\kappa$ in Equation 12.6 is then in nanometers. In milk, with an ionic strength $I$ of about 0.08 $M$, we find $1/\kappa$ equal to about 1.1 nm. Note that the pH largely determines the surface potential of a certain particle, and the ionic strength determines the decrease of potential with distance, hence the distance at which the repulsion works. Examples are given in Figure 12.3. At high $I$, $|\psi_o|$ also may decrease because counterions may become associated with ionized groups on the particle, thus neutralizing them.

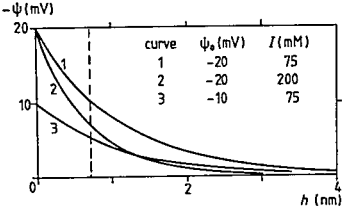



**Figure 12.3.** Effect of surface potential ($\psi_0$) and ionic strength ($I$) on the potential ($\psi$) at a distance ($h$) of a charged particle The electrokinetic (zeta) potential depends on the (mostly unknown) location of the slipping plane along the $h$ axis e g as indicated with the broken line

The thickness of the double layer has another important effect If the distances between charged groups (or clusters of groups) at a surface are $<< 1/\kappa$, then the charge may be considered to be evenly smeared out over the surface, even when both positive and negative charges are present But if the distance between charges is greater than $1/\kappa$, separate charged spots can be distinguished, this implies, for instance, that positive and negative groups attract each other and may form internal salt bridges Because of these effects, ionic strength may affect profoundly the conformation of charged macromolecules (macroions), like some proteins When $I$ is high, hence $1/\kappa$ small, the macroion tends to attain a more compact conformation

The ionic strength is usually easy to estimate, but the surface potential is not We can measure the electrokinetic or zeta potential ($\psi_\zeta$) by letting the particles move in an electric field But they move while taking at least a little solvent with them, so that the slipping plane, at which the potential is measured, is at some unknown distance from the surface (see Figure 12 3), hence $|\psi_\zeta|$ is less than $|\psi_0|$ Figure 12 4 gives some results for $\psi_\zeta$ for particles in milk

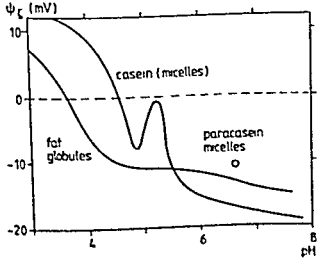



**Figure 12.4** Zeta potential ($\psi$) of particles from milk at various pH Approximate values at room temperature After various sources

### 12.2.3. DLVO Theory

The DLVO theory (Deryagin, Landau, Verwey, Overbeek) considers the combined effects of electrostatic repulsion and Van der Waals attraction. Usually, the energy needed to bring two particles from infinite distance to a mutual separation $h$ is calculated (as in Figure 12.5). For two spheres of radius $r$ and for conditions as in milk (the solvent is water, $\kappa r >> 1$) we find for the electrostatic interaction energy

$$V_R \approx 4.3 \times 10^{-10}\, r\, \psi_0^2 \ln(1 + e^{-\kappa h}) \qquad (12.7a)$$

expressed in SI units, hence $V$ in J. For the same conditions, and $h << r$, we find for the energy attributable to Van der Waals attraction

$$V_A \approx -Ar/12h \qquad (12.7b)$$

The total interaction is

$$V_T = V_R + V_A \qquad (12.7c)$$

It usually is compared to $kT$ ($\approx 4 \times 10^{-21}$ J). An example, calculated for milk fat globules, is in Figure 12.5.

The difficulty is to find the Hamaker constant $A$. This depends on the difference in polarizability between the material in the particles and in the continuous phase. Consequently, $A$ is low (e.g., $10^{-21}$ J) for swollen protein particles, because they consist largely of solvent. For fat globules in milk, $A$ is probably about $1.3 \times 10^{-20}$ J. But the membrane of the globules has a different, probably lower, Hamaker constant, and this affects the total attractive energy considerably.

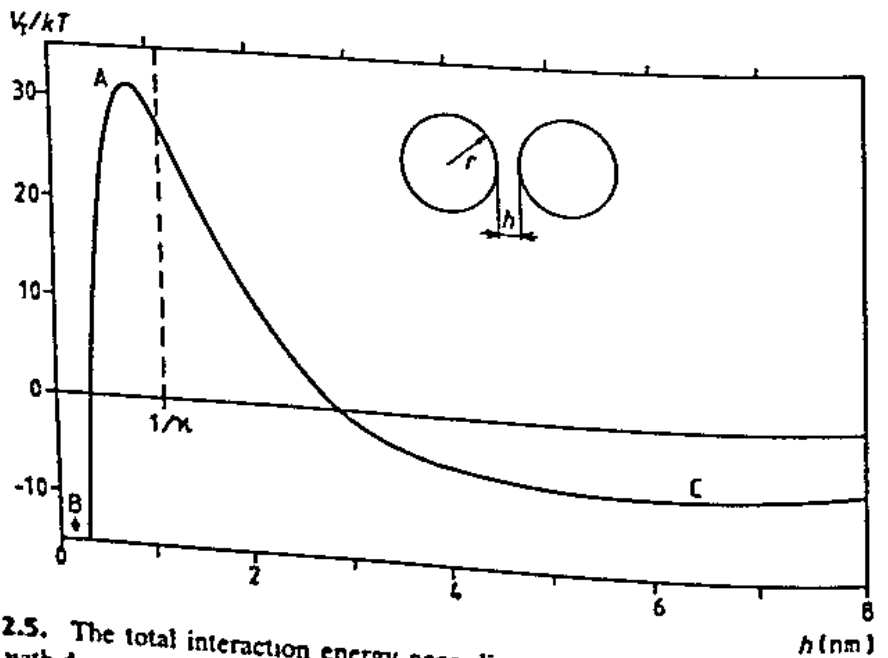



**Figure 12.5.** The total interaction energy according to Equation 12.7, $V_T$ between two fat globules with diameter $(2r) = 3$ μm as a function of distance $h$, for certain assumptions on the Hamaker constant and the surface potential

It should be emphasized that Equation 12 7 is a simplification and its parameters are uncertain Nevertheless, a curve similar to that in Figure 12 5 is found often If the maximum in the curve near A ($V_{max}$) is sufficiently high (say, 20 $kT$), the particles will not come into contact ($h = 0$) This is because the probability of two particles meeting to overcome this energy barrier is very low If $V_{max}$ is lower, the particles may come into contact or flocculate in the "primary minimum" B If they are fluid, this leads mostly to coalescence If they are solid, they remain together for a long time, unless $V_{max}$ is very low In most cases there is a "secondary minimum" near C, if it is deeper than, say, 3 $kT$, particles will flocculate in the secondary minimum (i e , at some distance from each other) Equation 12 7 shows that for a smaller $1/\kappa$, hence higher ionic strength, this minimum will be deeper

Although the DLVO theory adequately predicts the stability of many colloids, there is a serious difficulty in our cases Equation 12 7 shows that $V_T$ is proportional to $r$ Particle size does not affect the positions ($h$) of the maximum and secondary minimum, but rather it affects their magnitude This implies either that for small particles $V_{max}$ is too low to prevent flocculation in the primary minimum, or that for large particles the secondary minimum is so deep as to cause flocculation, this reasoning applies if the spread in particle size is sufficiently wide For milk fat globules, for instance, $r$ is between 0 1–8 0 $\mu$m Figure 12 5 indicates that for larger globules flocculation near C always should occur This also would be true if surface potential or Hamaker constant were somewhat different Nevertheless, large milk fat globules do not flocculate They do not flocculate even at their isoelectric pH (see Figure 12 3), where surface potential is zero (when this is investigated, casein should be absent)

### 12.2.4. Steric Repulsion

From the surface of a particle, molecular chains may protrude, and this can cause repulsion by about the same mechanism as macromolecules themselves are kept in solution If the particles approach closely, the layers with protruding chains (often called hairs) interpenetrate or become compressed, this causes loss of conformational entropy and thus repulsion If the hairs are hydrophilic, there is also repulsion by hydration on close contact of individual molecular chains The total effect is called steric repulsion, and its magnitude may be considerable, on the order of a few $kT$ per molecular chain (It also can be described in terms of an osmotic effect, arising from the increased concentration of macromolecular segments in the thin film between the particles )

Steric repulsion must be important for the stability of fat globules (Chapter 14) and casein micelles (Section 13 2) This implies that these particles are "hairy " Note that it will not prevent the particles from making contact The hairs may occasionally touch, and may even react chemically, if conditions are favorable for this In this way cross linking (e g , by –S–S–

bridging of protein) is still possible without the particles originally being in a flocculated state.

The hairiness may cause the zeta potential to be much lower than the surface potential, because the slipping plane of a particle moving through the liquid is further away from the surface. On the other hand, the hairs may carry charges themselves. All of these complications interfere with the precise calculation of the interaction energy between particles.

## 12.3. CHANGES IN DISPERSITY

Colloidal particles may change in size (by growth or dissolution and by coalescence or disruption) and in arrangement (by flocculation and by sedimentation or the inverse processes). Some aspects are discussed elsewhere (Ostwald ripening in Section 12.1.3; creaming in Section 14.5).

### 12.3.1 Flocculation Kinetics

When a liquid is at rest, particles in it meet each other because of Brownian motion. Smoluchovski's equation states for equal-sized spheres the number $J$ of encounters per unit volume and time to be

$$J_B = \frac{4}{3} kT N^2/\eta \tag{12.8}$$

where $N$ is the number of particles per unit volume and $\eta$ is the viscosity of the continuous phase. If an encounter leads to lasting contact, the particles are said to be flocculated and the number of particles decreases. If every encounter would lead to flocculation, the time $t_{0.5}$ needed to halve the number is, as derived from Equation 12.8, in water at room temperature

$$t_{0.5} \approx \frac{2.10^{17}}{N} \tag{12.8a}$$

where $N$ is in $m^{-3}$ and $t$ is in seconds. This would imply for the fat globules in milk $t_{0.5}$ of about 10 s and for the casein micelles $t_{0.5}$ of about 1 ms. Obviously, such a flocculation normally does not happen.

Flocculation may be retarded by two mechanisms. First, the liquid must flow away through the narrow gap between approaching particles. Unless these are very small, it causes considerable resistance, and the particles would never flocculate unless there were some attractive force at close approach (as near C in Figure 12.5). Second, and usually more important, an energy barrier or activation energy for flocculation may exist (as near A in Figure 12.5). This slows down flocculation by a factor of $\exp(-V_{max}/kT)$, or, for instance, $5 \times 10^{-5}$ for $V_{max} = 10\ kT$ and $2 \times 10^{-9}$ for $V_{max} = 20\ kT$.

When the liquid is not at rest, the meeting frequency of the particles may be much increased, as discussed in Section 12.3.3.

### 12.3.2. Gel Formation

Flocculation may lead either to a coagulum consisting of separate, compact flocs, which usually sediment and hence form a precipitate, or to a gel in which the flocculated particles form a network that extends througbout the liquid. Gelation, of course is preceded by formation of small floccules, and these, rather than separate particles, build up the network. If flocculation can go on unhindered, without any streaming in the liquid (e.g., as caused by stirring) and without appreciable sedimentation (or creaming) of the floccules, a gel is formed. Gelation is favored by the volume fraction of the particles being larger.

The casein micelles of milk usually form a gel on flocculation if the liquid is kept at rest, as in renneting, but when the liquid is stirred a coagulum forms. Aggregates of milk fat globules usually cream too fast for a gel to form, but in cream (e.g., 30% fat) this may happen.

A gel may show syneresis; this means that the network of particles shrinks, expelling solvent. An important example is discussed in Section 13.3.4.

### 12.3.3. Effects of Streaming

Milk and liquids derived from it often undergo streaming, particularly during processing, and that may affect the colloidal particles. Streaming of a liquid generally evokes a velocity gradient ($G$, expressed in $s^{-1}$); its magnitude largely determines the effects of streaming on particles. Very roughly, $G$ is $0.01\ s^{-1}$ for flow around a creaming fat globule, $1\ s^{-1}$ for convection currents in a beaker of milk being heated, $100\ s^{-1}$ for flow through a pipeline, and up to $10^4\ s^{-1}$ may be caused by mechanical stirring as applied in practice. In laminar streaming, $G$ is roughly constant in fairly large regions of the liquid. In turbulent streaming, $G$ varies widely with place and time, and inertial effects become important; some aspects are discussed in relation to homogenization. (See Section 14.4.1.) Here we will restrict ourselves to the simpler case of laminar flow.

Streaming may affect the meeting frequency of colloidal particles. Instead of Equation 12.8 we now have (for equal-sized spheres)

$$J_G = \frac{2}{3} d^3 N^2 G = 4\,\phi\, N\, G/\pi \qquad (12.9)$$

Note the strong dependence on particle size and the absence of viscosity in the equation. For particles in water at room temperature, we obtain for the ratio of gradient to Brownian meeting frequency

$$\frac{J_G}{J_B} \approx 0.2\, d^3 G \qquad (12.10)$$

when $d$ is expressed in micrometers. Consequently, for milk fat globules often $J_G >> J_B$, for casein micelles usually $J_B >> J_G$.

Streaming exerts a viscous stress on particles in the liquid, causing them to deform and often to rotate. The stress is on the order of $\eta G$, where $\eta$ is the viscosity of the surrounding liquid (roughly $10^{-3}$ Pa·s for milk plasma). For a fluid particle, the stress is counteracted by the Laplace pressure, $4\gamma/d$ (Equation 12.2). If the ratio of viscous stress to Laplace pressure is sufficiently large, the particle may deform to such an extent that it is disrupted into smaller particles; the condition is roughly $\frac{G\eta d}{\gamma} > 0.5$. Consequently, milk fat globules ($\gamma \approx 2.5$ mN·m$^{-1}$) can be disrupted only by velocity gradients of $10^5$–$10^6$ s$^{-1}$. Such high gradients are very difficult to achieve and may occur where a fast-flowing liquid impinges against an obstacle or near a rotor blade going at high speed through the liquid; they also can occur during atomization of milk, as applied during spray drying. Particles smaller than fat globules generally are not affected at all. Air bubbles of $d = 2$ mm in milk require $G \approx 10^4$ s$^{-1}$ for disruption, and that is certainly possible during beating.

Streaming also exerts a viscous stress on flocs of particles and may disrupt them into smaller floccules or single particles if the stress can overcome the attractive forces between the particles; likewise, streaming may prevent floccules from forming. Generally, large flocs are easier to disrupt than small ones, because they are more likely to have relatively weak spots. Flocs of milk fat globules formed by cold agglutination (Section 14.3) can be disrupted completely if $G \approx 500$ s$^{-1}$. Flocs of casein micelles (e.g., formed by renneting or acidification) cannot be disrupted so easily; sizable floccules remain even when the liquid is vigorously stirred during the flocculation process.

## 12.4. SIZE DISTRIBUTIONS

The colloidal particles of milk show a wide range of sizes. Several properties of the particles depend on their size. Parameters characterizing the size distribution are discussed here but restricted to the relatively simple case of spherical particles, which is adequate for casein micelles and fat globules.

For the sake of explanation we divide the distribution into size classes where the $i$th class contains particles with diameters between $d_i - \frac{1}{2}\Delta d$ and $d_i + \frac{1}{2}\Delta d$; the number of particles per unit volume in the size class is $N_i$. Now the number frequency is given by $N_i/\Delta d$. Note that number frequency is a differential quantity with the dimension of length$^{-4}$, number per unit volume per unit class width. The volume frequency, which gives the amount of material present in particles of a certain diameter, is obtained by multiplying number frequency by the volume of a particle $\pi d_i^3/6$; it is thus $\pi N_i d_i^3/6\Delta d$. We also may express it as percentage of the total volume of material per unit class width; it is then equal to the mass frequency if dif-

**Table 12.2. Parameters of Size Distributions**

| Parameter | Symbol | | Formula | Dimension | Equation |
|---|---|---|---|---|---|
| $n$th moment of distribution | $S_n$ | = | $\Sigma N_i d_i^n$ | $[L^{n-3}]$ | (12 11) |
| Number average $d$ | $\bar{d}$ | = | $S_1/S_0$ | [L] | (12 12) |
| Volume/surface average $d$ | $d_{vs}$ | = | $S_3/S_2$ | [L] | (12 13) |
| Stokes parameter | $H$ | = | $S_5/S_3$ | $[L^2]$ | (12 14) |
| Relative width | $\sigma_s$ | = | $(S_2S_4/S_3^2 - 1)^{\frac{1}{2}}$ | — | (12 15) |
| Volume fraction | $\phi$ | = | $\pi S_3$ | — | (12 16) |
| Specific surface area | $A$ | = | $\pi S_2 = 6\phi/d_{vs}$ | $[L^{-1}]$ | (12 17) |
| Mean free distance | $z$ | = | $0\ 225 d_{vs}(0\ 74/\phi - 1)$ | [L] | (12 18) |

*Note* $N_i/\Delta d$ is the number frequency $d$ is particle diameter Only for spherical particles

ferences in density of the particles are negligible Examples are in Figures 13 2 and 14 1 If $\Delta d$ is made infinitely small, a smooth curve of frequency versus diameter is obtained

Table 12 2 gives some parameters of size distributions The moments of the distribution $S_n$ are auxiliary parameters, simplifying equations and calculations Numerous types of average diameters can be calculated from the general equation $\left(\frac{S_n}{S_m}\right)^{1/(n-m)}$ Usually, the arithmetic or number average $d_{10}$ or $\bar{d}$ is taken, but it is not a very useful average for casein micelles or fat globules because of the very large number of relatively small particles This implies that $\bar{d}$ can hardly be determined with accuracy, the total number of globules ($S_0$) is difficult to obtain The volume–surface average $d_{32}$ or $d_{vs}$ is more useful We also may define it as the surface-weighted average It relates the total volume of dispersed material to its total surface area (Equation 12 17), both important parameters It recurs in the equation for the mean free distance (Equation 12 18), which is the average linear distance a particle can move before touching another Mean free distance will be used in Section 14 2 The Stokes parameter will be discussed in Section 14 5 in relation to creaming Volume fraction and specific surface area are self-explanatory

A size distribution is not defined by its average A second important parameter is the width, usually expressed as standard deviation The relative standard deviation (obtained by dividing by the average size) is the most meaningful measure of polydispersity Equation 12 15 gives the surface-weighted relative width $\sigma_s$, corresponding to $d_{vs}$

## *Suggested Literature*

Introductory textbooks in colloid and surface science are

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K. J. Mysels, *Introduction to Colloid Chemistry*, (New York: John Wiley, 1959).

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# 13

# Casein Micelles

The casein micelles of cow's milk are intricate particles that carry most of its protein and a considerable portion of its Ca and phosphate Their primary function is to provide the calf with these important nutrients The difficulty in the "design" of the micelles is that they must contain some highly insoluble material (the calcium phosphate) while being themselves stable and not too large On the other hand, they form a clot once in the stomach of the calf, and it must be assumed that the clotting also is essential These somewhat conflicting limiting conditions may account for the intricacy of the micelles

The existence and the properties of casein micelles have several consequences in the use of milk To a large extent they determine the stability of milk products during heating, concentrating, and holding Their behavior is essential in various steps of cheese making The micelles and the changes occurring in them largely determine the rheological properties of sour and concentrated milk products Casein micelles interact with air–water and oil–water interfaces, the latter interaction is particularly important in homogenization and affects some properties (stability, viscosity) of homogenized products

## 13.1. PROPERTIES

Almost all casein in uncooled milk is present in roughly spherical particles, mostly 0 02 to 0 30 $\mu$m in diameter, comprised of some 20–150,000 casein molecules These casein micelles also contain inorganic matter, mainly calcium phosphate, about 8 g per 100 g casein (See table 4 2 ) The particles are voluminous, holding more water than casein Finally, they contain small quantities of some other proteins, such as the milk enzymes lipase and plasmin and part of the proteose–peptone

What is the *structure* of the casein micelles? When whole casein is in solution at a concentration, pH, and ionic strength as in milk, but at low $Ca^{2+}$ activity, small aggregates form. There is no agreement on their size; diameters of 10–20 nm and molecular weights of 250,000–2,000,000 have been reported. Hence, they contain something between ten and one hundred casein molecules. The aggregates, like globular proteins, will have a dense hydrophobic core, in which most hydrophobic parts of the casein molecules are buried, and a more loosely packed, hydrophilic outer layer, containing most of the acidic (carboxylic and phosphoric) and some of the basic groups. The association of casein into such aggregates bears some resemblance to soap micelles, which implies an equilibrium between free and associated molecules that is far to the associated side.

Each of these small aggregates of whole casein, usually called submicelles, contains different casein molecules. However, they cannot be identical in composition, considering the molar ratio of the principal caseins (about $\alpha_{s1}$:($\beta$+$\gamma$):$\kappa$:$\alpha_{s2}$ = 8:8:3:2); the variability of their ratio, particularly of $\kappa$-casein; and the differences in glycosylation and phosphorylation of $\kappa$- and $\alpha_{s2}$-caseins, respectively. Moreover, $\kappa$-casein probably exists in milk as an oligomer, containing several molecules and held together by covalent bonds (–S–S– linkages). Consequently, there may well be essentially two types of submicelles, with and without $\kappa$-casein.

On increasing the activity of $Ca^{2+}$ the association increases considerably. Under conditions as in milk—which means in particular that besides Ca, phosphate is present—the submicelles aggregate into micelles. (Note that the word *micelle* is used here in the wide sense of a small aggregate of molecules, and not in the much stricter sense common in colloid science.) The calcium phosphate acts as a cementing agent, but it is not known what bonds are involved. The aggregation would go on until a gel or a precipitate was formed if there were no $\kappa$-casein, which thus acts as a "protective colloid". The C-terminal part of $\kappa$-casein is very hydrophilic, particularly those molecules that contain carbohydrate, and it also has a considerable negative charge. Presumably, this part of the molecule sticks partly out into the surrounding medium as a flexible "hair," more or less behaving as a random-coil polymer chain. Consequently, a submicelle with $\kappa$-casein would exhibit steric repulsion at least at part of its surface (Section 12.2.4), and aggregation at such a site would be greatly hindered, if not prevented. In this way, aggregation of submicelles would go on until the surface of the micelle was more or less covered with $\kappa$-casein. This does not imply that there is only $\kappa$-casein at the surface; the $\kappa$-casein will be interspersed with other caseins.

Figure 13.1 depicts the model of the casein micelle deriving from the reasoning above. Most of the $\kappa$-casein is at the outside, and the protruding chains of its C-terminal end give the micelle a hairy surface (though the hairs are too thin to be observable with electron microscopy). The hairs are flexible and show perpetual Brownian motion. The effective thickness of the hairy layer is at least 5 nm. A small part of the $\kappa$-casein is in the interior.

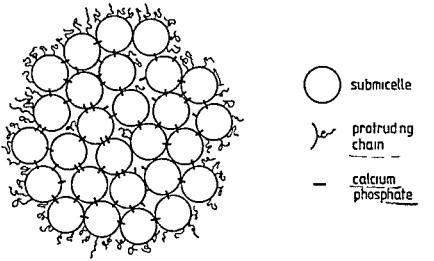



**Figure 13.1.** Model of a casein micelle, highly schematic Mainly after D G Schmidt and T A J Payens, *Surface Colloid Sci* 9 (1976) 165, W Slattery *Biophys Chem* 6 1977) 59, and P Walstra, *J Dairy Res* 46 (1979) 317

The micelle is fairly voluminous, but this term may be interpreted in various ways We will consider first the volume of the particles without the hairy layer Electron micrographs indicate that the voluminosity $\nu$ is about 2 ml per gram of casein About half of that volume may be in the submicelles, the rest is water, or rather serum, between the submicelles The serum inside the micelles, however, is depleted of part of the large solute molecules (See Section 17 2 2 ) The pore width in the micelle is probably a few nanometers, permitting easy access by many solute molecules but restricting the access of globular proteins Taking the hairy outer layer into consideration, micelle voluminosity is higher, on average about 4 ml per gram of casein It can be determined from hydrodynamic measurements (e g , from intrinsic viscosity) The hairy layer is included and has a constant thickness, so hydrodynamic voluminosity increases with decreasing micelle size

The *size distribution* and some derived data are given in Figure 13 2 and Table 13 1 See Section 12 4 for general aspects of size distributions Some

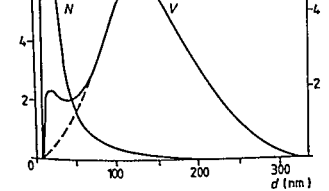



**Figure 13 2.** Example of the size frequency distribution of casein micelles Number (*N*, left hand ordinate) and volume frequency (*V*, right hand ordinate) both in percentage of total per 50 nm class width, against micelle diameter (*d*) After D G Schmidt P Walstra and W Buchheim *Neth Milk Dairy J* 27 (1973) 128

**Table 13.1. Size Distribution of Casein Micelles**

| Parameter | Full Distribution | Without Particles < 20 nm |
|---|---|---|
| Number per $\mu m^3$ milk | 600 | 120 |
| Volume fraction ($\phi$) | 0.06[a] | |
| Number average diameter $\bar{d}$ (nm) | 25 | 65 |
| Volume–surface average, $d_{vs}$ (nm) | 86 | 104 |
| Relative width[b] | 0.4 | |
| Mean free distance $z$ (nm) | 240 | 290 |
| Surface area ($m^2 \cdot ml^{-1}$ milk) | 4.0 | 3.3 |
| Same of submicelles | 8.–17.? | |

*Note:* Approximate values of some parameters.
Source: Mainly after D. G. Schmidt, P. Walstra, and W. Buchheim, *Neth. Milk Dairy J.* 27 (1973):128
[a]Including the hairy layer ~ 0.12.
[b]Standard deviation/average of the volume distribution.

investigators have found a small number of very large micelles, up to 800 nm diameter. The large number of very small particles concerns mainly loose submicelles; they comprise 1–2% of the total casein at room temperature.

The casein micelles are not static. We may conceive three *dynamic equilibria* between the micelle and its surroundings:

1. Between free casein molecules and submicelles. A tiny part of the casein is in true solution, but precisely how much is not known because it is difficult to separate dissolved casein from loose submicelles. The equilibrium depends strongly on temperature. (See below.) The relaxation time (i.e., the time needed to reduce a small deviation from the equilibrium to $1/e = 37\%$ of its original value) appears to range from several hours at 0°C to about 10 min at 50°C; these times depend very much on conditions, however. Moreover, it is not certain that casein molecules, which leave the micelles at low temperature, will return in the same position or configuration they had before.
2. Between dissolved and colloidal Ca and phosphate. The existence of this dynamic equilibrium is a prerequisite for that of the next one. But the situation is complicated, and there may be several equilibria, each with its own relaxation time.
3. Between free submicelles and micelles. The relaxation time is at least a few minutes at room temperature. The association equilibrium of the submicelles, hence the size distribution of the micelles, is determined primarily by the relative quantity of $\kappa$-casein, but other factors, notably content of calcium phosphate, also affect it. Mixing of milks

with different-sized casein micelles, diluting milk with its ultrafiltrate, and changes in the salt composition of the serum (e g , pH, $[Ca^{2+}]$) lead to (mostly small) changes in the size distribution Temperature also affects micelle size, as discussed below

The model of the casein micelle as outlined here is to some extent conjectural We consider it the model in best agreement with the numerous observations on properties and stability of the casein micelles, including renneting Several aspects of it are almost common ground among researchers But other features, like the existence of two major kinds of submicelles and the hairiness of the micelle, are a matter of debate, while the size of the submicelles and the relaxation times of the equilibria are still uncertain We will not discuss these problems at length in this book

The casein micelles are rather variable, particularly in milk of individual cows Casein composition varies, though not very much (See Section 6 3 ) The proportion of $\kappa$-casein varies from about 9–15%, and this will be reflected in average casein micelle size, which may vary by perhaps a factor 1 4 Voluminosity varies by nearly a factor of 2 Variations in size and voluminosity may be caused partly by variation in the content of inorganic matter, usually from 6–9 g per 100 g dry casein Composition of inorganic matter may vary, too (See Section 4 4 ) Between micelles of one milking of one cow, the content of $\kappa$ casein (and even more of N-acetyl neuraminic acid) markedly decreases with increasing micelle size, while the content of calcium phosphate slightly increases with size

The inorganic matter of the casein micelles is discussed in Section 4 4 Suffice it here to state that it can be thought to be made up of counterions ($K^+$, $Na^+$, most of the $Mg^{2+}$, and part of the $Ca^{2+}$) and colloidal calcium phosphate, an amorphous salt The latter has a molar ratio Ca/P $\approx$ 1 5–1 6, and it also contains some citrate and possibly Mg It is bound to and stabilized by the casein The quantity of calcium phosphate in the micelles increases on heating (Section 4 5) and very much decreases when lowering the pH

The casein micelles will be *dispersed into smaller units* by any treatment that dissolves a considerable proportion of the calcium phosphate at constant pH, such as adding a Ca binder (like sodium citrate) or dialyzing against a Ca-free solution Small reductions in colloidal calcium phosphate do not cause significant changes in micelle size, though there may be both a partial disintegration and an increase in voluminosity of the micelles, leaving their average size about the same Adding NaCl (i e , raising the $Na^+/Ca^{2+}$ ratio and the ionic strength) also leads to partial disintegration All of these changes can be reversed largely by bringing the milk back to its original composition Raising the pH (e g , with NaOH) causes calcium phosphate to precipitate, and the micelles partially disintegrate Micelles disperse into submicelles when milk is subjected to high pressure (e g , 100 MPa for 1 h), this disintegration is irreversible, and it may be caused by the formation of hydroxyapatite from the calcium phosphate

The casein micelles also will dissolve when the submicelles dissociate. The molecules in the latter are held together mostly by hydrophobic interaction and by H bonds in the hydrophobic interior of the submicelle. Consequently, addition of large quantities of urea or guanidinium chloride dissolve the micelles, and much smaller quantities of sodium dodecyl sulfate do the same. Electrostatic interactions (i.e., internal salt bridges) also participate in keeping the submicelles together, but they cannot be broken without dissolving the calcium phosphate. Reagents that break –S–S– linkages do not disintegrate the micelles fully, as is to be expected, but it is not known whether less rigorous changes occur.

*Lowering the temperature* (e.g., to 5°C) considerably affects the casein micelles. Hydrophobic interaction becomes much weaker, and part of the casein, particularly of the β-casein, dissociates from the micelles. The voluminosity of the micelles increases, probably in part from increased "hairiness," as β-casein chains may protrude from the micelle surface. A small part of the calcium phosphate dissolves. (See Section 4.5.) These changes may be the cause of the slight disintegration of the micelles; despite the increase in voluminosity, their average size decreases somewhat (e.g., by 15%).

## 13.2. COLLOIDAL STABILITY

The stability of casein micelles is a somewhat confusing subject. Several different treatments may lead to aggregation of the micelles. If the cause if a change in environment, such as pH or salt content, the lack of stability usually can be explained in terms of colloid science; the aggregation can be reversed by restoring the original environment. Aggregation also may be a sequel to a chemical change in the casein micelles; examples are renneting (Section 13.3), age gelation (Section 13.4), and heat coagulation (Section 13.5). In these cases, the aggregation is usually irreversible. Still, the colloidal aspects also need to be considered, as they often interact with other factors in the ultimate stability.

Another problem lies in the definition of stability. Some treatments cause a (partial) dissolution of the casein micelles and may make the milk more stable, but it cannot be stated that the micelles have become so. Other treatments may cause casein micelles to fuse into larger ones; often there is no outward indication of a decreased stability, but a slight change in conditions now may cause the micelles to form a precipitate or a gel.

A third confusing aspect lies in the many factors or treatments that affect the stability and in the fact that most treatments cause more than one change in the micelles. Consequently, a quantitative explanation of casein micelle stability cannot be given.

A caseinate solution is comparatively stable. Boiling, for instance, causes no significant change. Whole casein precipitates at its isoelectric pH (≈4.6),

it loses its net charge and forms internal salt bridges, and its high hydrophobicity then will make the casein insoluble Casein can be salted out, like most proteins It is relatively sensitive to $Ca^{2+}$ ions, and a $Ca^{2+}$ activity on the order of 50 m$M$ causes precipitation Probably, $Ca^{2+}$ makes the casein much more hydrophobic by screening the ester phosphate groups The stability depends much on casein composition, markedly increasing with increasing proportion of $\kappa$-casein See further Section 6 3, where the differences between the various caseins also are discussed

Casein micelles are less stable than caseinate solutions They are relatively large particles, and Van der Waals attraction becomes important The decrease in conformational entropy on aggregation is less than in the case of caseinate Still, the micelles are usually stable under conditions as in fresh milk When they are sedimented by high-speed centrifuging, they form a gel like pellet, but this gel can be redispersed again in the supernatant

The stability of the micelles usually correlates well with their voluminosity $v$ This may be because a higher $v$ may correspond with a more extensive hairiness, hence to a stronger steric repulsion (See Section 12 2 4 ) But $v$ also may be correlated with the compactness of the micelle core, and Van der Waals attraction (Section 12 2 3) then will increase strongly with decreasing $v$, because the Hamaker constant is in first approximation proportional to $v^{-2}$ Taking the change in radius also into account, the attraction energy would be proportional to $v^{-5/3}$ Another factor in micelle stability is presumably electrostatic repulsion The zeta potential is, for instance $-13$ mV at 20°C and pH 6 7, its absolute value increases with temperature (e g , 0 3 mV $K^{-1}$) and with pH (e g , 0 4 mV per unit)

Without calcium phosphate, casein submicelles do not form micelles, and the latter are generally less stable if they contain more calcium phosphate This may be the result of the correlation with micelle size and voluminosity Generally, a high citrate content of the milk correlates with a low content of calcium phosphate in the micelles In some regions a cow occasionally may give milk that spontaneously curdles after milking, the so called Utrecht milk abnormality It is probably related to an excessive concentration of colloidal calcium phosphate

There are considerable differences in stability among lots of milk, and there are effects of season (perhaps stage of lactation) and individual cows Differences in casein composition, content of calcium phosphate, micelle size and voluminosity, and $Ca^{2+}$ activity all may play a part, these variables, of course, are correlated to some extent

Several factors influence casein micelle stability

1. Salt composition affects $Ca^{2+}$ activity of the serum and calcium phosphate content of the micelles These variables are closely related, and it is not known which of them is the more important factor affecting stability Examples of the effect of various additions are in Table 4 5 Addition of $CaCl_2$, for instance, raises $Ca^{2+}$ activity and

micellar calcium phosphate and decreases pH, all of them being detrimental to stability. Adding NaCl will increase ionic strength somewhat and decrease calcium phosphate content of the micelles; it usually increases stability. Sodium citrate addition enhances stability. Sodium phosphate may work either way: It lowers $Ca^{2+}$ activity, increases phosphate in the micelles, and may affect pH.

2. Lowering the pH leads at first to dissolution of colloidal calcium phosphate (Figure 4.1) and a decrease in micelle voluminosity; some dissolution of micelles may also occur. A still lower pH (< 5.5) leads to enlargement of casein micelles, as micelles fuse. This may be caused by loss of surface potential. Figure 12.4 shows that the (negative) zeta potential rises to about zero near pH 5.2. Most but not all of the colloidal phosphate is now lost. At still lower pH more phosphate dissolves, and the casein starts to precipitate, though zeta potential falls again. Presumably, the colloidal calcium phosphate is positively charged in this region, while the casein still has a net negative charge. Near pH 4.8 almost all phosphate is dissolved, and near pH 4.6, the isoelectric point, the solubility of casein is negligible. Now, casein rather than casein micelles precipitates. Acid precipitation is applied commonly to collect casein from skim milk.
3. Temperature has a large effect. Near 0°C, aggregation of casein micelles is very difficult to achieve. Aggregation rate (for conditions where the micelles are unstable) has a $Q_{10}$ of 2–5, according to type of instability and other conditions. Lowering the temperature increases voluminosity, probably increases steric repulsion ($\beta$-casein chains now may protrude from the micelle surface), slightly increases $Ca^{2+}$ activity, somewhat decreases calcium phosphate in the micelles (as solubility increases), and somewhat decreases average micelle size. For increasing temperature these trends are reversed. Temperature also affects the nature of the gel or precipitate formed by acidification. Lower temperatures give a finer, more voluminous precipitate (when the milk is stirred during acidification), and a weaker gel (when the milk is kept still).
4. Heat treatment of the milk to such an extent that the greater part of the whey proteins become associated with the casein micelles (Chapter 10) alters the behavior of the micelles, for instance, on acidification. Fusing of micelles below pH 5.5 is far less, and the gel obtained near pH 4.5 is firmer. Other effects of heat treatment on properties of casein micelles are discussed in Section 13.4 and 13.5; they cannot be explained satisfactorily.
5. Dehydration, for instance, by adding ethanol, leads to aggregation of the micelles. Presumably, the solvent quality for the protruding chains of $\kappa$-casein is lowered, causing a decrease in voluminosity and in steric repulsion. Ethanol to a concentration of 50–70% can be added to normal fresh milk before coagulation is observed. A similar effect

occurs on adding large quantities of salts. The aggregation of casein micelles in frozen milk products also seems to be caused by salting out. (See Section 17.4.)

Many of the effects causing instability are additive, for instance, low pH and ethanol. This principle has been applied in the alcohol stability test to detect sour milk; the lower the pH, the lower the ethanol concentration needed to cause coagulation. Another example is the decreased micelle stability in concentrated milk. Removal of water leads to a lower pH (hence a loss of surface potential), a higher $Ca^{2+}$ activity, a higher ionic strength (hence a lower solvent quality and a thinner diffuse double layer), and a higher concentration of colloidal calcium phosphate in the micelles. All of these factors lessen stability. In evaporated milk the micelles have become much larger (up to a new micrometers in diameter), and further concentration eventually leads to gel formation.

Casein micelles may interact with other substances, affecting their stability:

1. Some hydrocolloids, particularly κ-carrageenan, bind to casein micelles. Electrostatic interaction of the negative carrageenan with positively charged regions of κ- and $\alpha_{s2}$-casein is involved. (See also Section 16.2.) Carboxymethyl cellulose interacts, possibly in a similar way, at pH 7.5. By adding an excess of such hydrocolloids, the casein can be precipitated and separated from skim milk.
2. Most surfactants bind to proteins, largely through hydrophobic interaction. Small quantities of nonionic surfactants (< 5 mol per mole of casein) have little effect, but cationic ones (e.g., quaternary ammonium compounds) decrease the absolute value of the zeta potential of the micelles, thereby somewhat decreasing their stability. Anionic surfactants (e.g., sodium dodecyl sulfate) have the opposite effect; free fatty acids do the same, but they also may enhance stability by binding $Ca^{2+}$. Many surfactants, when present in high concentration, disintegrate protein aggregates, hence casein micelles.
3. Casein micelles may interact with oil–water and air–water interfaces, for instance, during homogenization and foaming. Some interaction with fat globules is almost unavoidable; consequently, casein preparations always contain some fat (say, 1%), even after washing.

## 13.3. RENNETING

Many proteolytic enzymes are able to clot milk; this means that the milk forms a gel some time after a preparation containing the enzyme has been added. The most used preparation is calf rennet, the active principle of which is chymosin.

Two stages can be distinguished in clotting or renneting:

1. casein $\xrightarrow{enzyme}$ paracasein + whey proteose
2. paracasein micelles $\xrightarrow{Ca^{2+}}$ gel

Renneting is the essential step in the manufacture of most types of cheese. During cheese making there is usually a simultaneous production of lactic acid by starter organisms. This affects the clotting reaction as well as many other changes occurring later.

### 13.3.1. Chymosin

Chymosin, sometimes called rennin, is an endopeptidase—E.C. 3.4.23.4. MW 30,700, isoelectric pH 4.6–4.7. It is readily soluble in water. In the abomasum (fourth stomach) of young calves a precursor, called prochymosin or prorennin, is synthesized. In an acid environment a peptide of some 5300 MW is split off, leaving chymosin; this cleavage is to some extent autocatalytic (i.e., the enzyme itself splits off the peptide). As the calf becomes older, its abomasum produces more and more pepsin (E.C. 3.4.23.1) and less and less chymosin. Bovine pepsin is very similar in action to chymosin; unlike chymosin, it can hydrolyze the immunoglobulins of colostrum. Commercial rennets always contain some pepsin.

Chymosin hydrolyzes protein molecules into large peptides. Some peptide bonds are split much more easily than others, and it is the sequence of amino acid residues in a certain part of the peptide chain, as well as the exposure of that part, that determines the lability of a bond to cleavage. The higher the pH, the fewer the bonds that can be split. At pH 6.7, it is only κ-casein, and just one bond in that, which can be split at a reasonable rate. (See below.) The optimal pH for overall proteolysis is about 3.8.

Chymosin is inactivated easily. At low pH this is an autocatalytic reaction. Inactivation at high pH is by a different mechanism, presumably denaturation. Figure 13.3 gives some variables. Salt inhibits inactivation, hence the high salt content of commercial rennets. In milk the inactivation depends much on temperature: $Q_{10} \approx 2.5$. Above 40°C, the enzyme may be inactivated before the milk clots.

Chymosin is adsorbed onto paracasein. At pH 6.7 and 33°C the adsorption is very weak, but it increases with decreasing temperature and pH. $Ca^{2+}$ ions enhance adsorption. The amount of rennet included in cheese thus depends on cheese-making practice.

The proteolytic action of clotting enzymes in cheese is of great importance for the ripening of many types of cheese. Proteolysis by chymosin decreases with increasing pH, but the optimum in cheese is much higher than the optimal pH of the enzyme when measured as initial rate on a soluble protein. This is because the amount of enzyme included, its inactivation, and the solubility of the substrate all depend on pH. The different caseins are split at different rates, and the sequence is $\alpha_{s1} > \beta > \alpha_{s2}$; κ-casein is a special

case. Salt inhibits the proteolytic action, particularly on β-casein. Undenatured whey proteins appear to be scarcely proteolyzed by chymosin. Pepsin can split more bonds of casein than chymosin at the same pH.

### 13.3.2. The Enzymatic Stage of Renneting

Chymosin (as well as many other endopeptidases) splits particularly the Phe–Met bond of κ-casein (i.e., the bond between phenylalanine residue 105 and methionine 106). In milk, at least 95% of the κ-casein is split. This is a relatively quick reaction, the turnover rate being on the order of 100 $s^{-1}$ (milk, pH 6.7, 30°C). Small peptides containing the same bond also are split, though the reaction is much slower than on κ-casein; the rate increases as the peptide is a larger portion of the κ-casein molecule. This implies that the part of the molecule containing the Phe–Met bond is exposed. Presumably, the positive charge of the region of the peptide chain (Figure 6.3) accounts for a rapid and easy binding of the negatively charged enzyme molecule. The reaction has a $Q_{10}$ of about 3. Around pH 6.6 its rate is about proportional to the $H^+$ ion activity; it is not affected by $Ca^{2+}$, but a certain ionic strength is needed. (See also Figure 13.3.)

κ-casein is split into para-κ-casein and a caseinomacropeptide. (The latter is thus identical to the whey proteose, at least when only chymosin acts for a short time at milk pH.) The two polypeptides have very different properties. (See Table 13.2.) The para-κ-casein is insoluble in milk serum and also in the absence of $Ca^{2+}$, but it can be kept in dispersion by the other caseins. The caseinomacropeptide is very soluble, and it does not associate; it is present in whey in an extended conformation, almost as a random coil. The caseinomacropeptide is heterogeneous because of the variation in glucide content. (See Section 6.3.5.)

The action of clotting enzymes in milk usually is followed by determining the amount of protein N becoming soluble. (See Figure 13.4.) Soluble peptides containing about 3.8% of the N are formed relatively quickly; this primary proteolysis pertains predominantly to splitting off the caseinomacropeptide. A much slower, pH-dependent secondary proteolysis follows, and it differs much among enzymes; this concerns not only the rate but also the peptides formed. The rate of secondary proteolysis has been used to assay the strength of rennet preparations, using sodium paracaseinate as the substrate. This works well for chymosin, but different enzymes usually have a different ratio of primary to secondary proteolysis. It is better and easier to use synthetic peptides and determine the rate of primary proteolysis. But even then the ratio of the rate of splitting of the peptide to that of primary proteolysis in casein may differ among enzymes.

Attempts have been made to split casein (primary proteolysis) by means of immobilized enzymes, but the results are inconsistent. We know of no experiments that clearly prove that normal clotting can be produced in milk by immobilized chymosin.

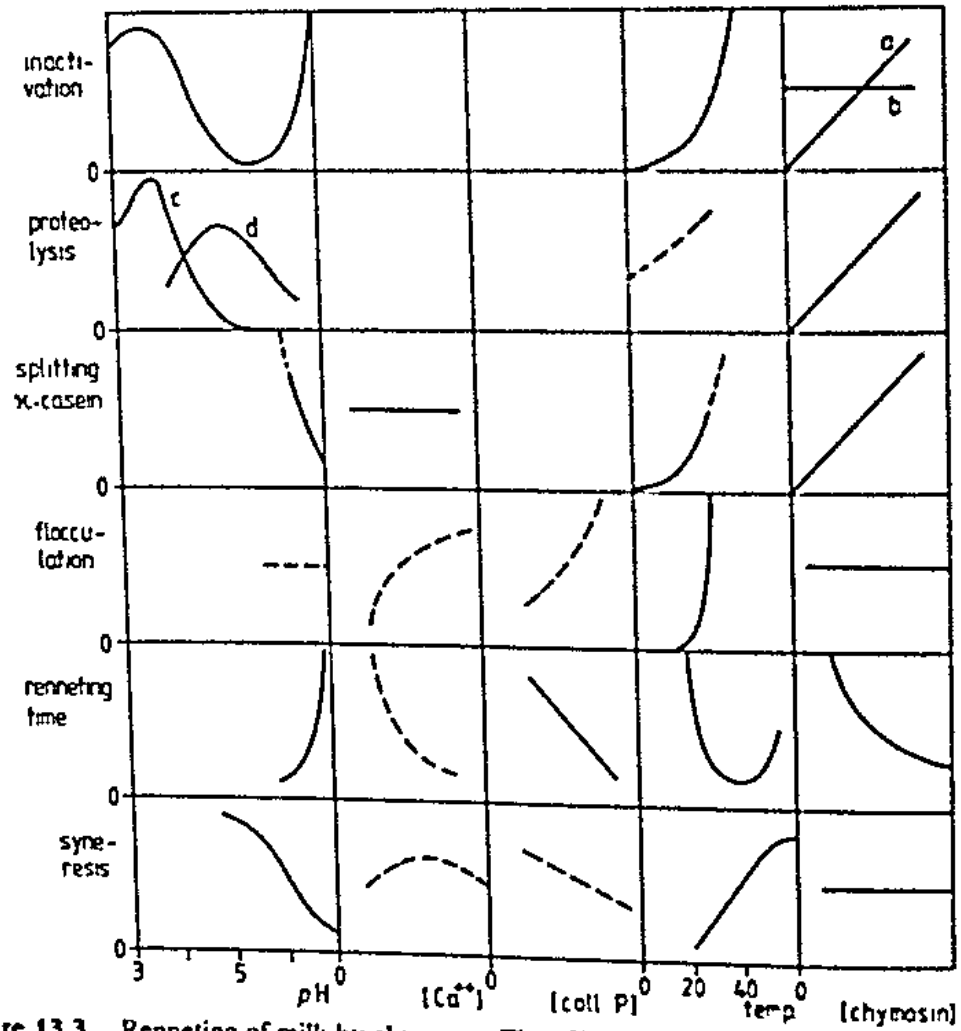



**Figure 13.3.** Renneting of milk by chymosin The effect of pH, $Ca^{2+}$ concentration, content of calcium phosphate in the micelles, temperature, and chymosin concentration on the rates of chymosin inactivation, proteolysis, splitting of κ-casein, and flocculation of paracasein micelles, and on renneting time and syneresis rate (a) At pH 3 5, (b) at pH 7, (c) during short times on soluble protein, (d) during, e g , one month in cheese Blank spaces imply no or little effect. Broken lines are rough estimates Only meant to illustrate trends

### 13.3.3. The Flocculation of Paracasein Micelles

Para-κ-casein has lost the protective-colloid property of κ-casein. Sodium paracaseinate (pH 6.7) precipitates at a $Ca^{2+}$ activity of about 7 m*M*. But in milk, the activity of $Ca^{2+}$ and $Mg^{2+}$ together is less than 2 m*M*. Consequently, the flocculation of para-casein micelles cannot be compared directly to the precipitation of paracaseinate.

The enzyme action does not destroy the micelles, though they do change in properties. Their average voluminosity (as determined from hydrodynamic measurements) is much reduced. The absolute value of the zeta potential decreases by about 40%. These changes must be a result of the loss of the protruding chains of κ-casein. They imply that repulsion between micelles is much diminished. The micelles may now approach each other closely and

**Table 13.2. Properties of κ-Casein and the Polypeptides Formed by Chymosin Action**

| Property | κ-Casein | Para-κ-Casein | Caseino-Macro peptide |
|---|---|---|---|
| Number of residues | 169 | 105 | 64 |
| *MW* peptide chain | 19,023 | 12,269 | 6,754 |
| *MW* inclusive glucide | ~19,550 | 12,269 | ~7,280 |
| Percentage glucide residues[a] | 1.4 | 0 | 3.6 |
| Number of positive charges | 15.5 | 12.5 | 4.0 |
| Number of negative charges | ~19.5 | 8.0 | ~12.5 |
| Hydrophobicity (kJ per residue) | 5.0 | 5.5 | 4.5 |
| Number of SerP residues | 1.(2.) | 0 | 1.(2.) |
| Number of –S–S– bridges | 1 | 1 | 0 |

[a]Average number of hexose and NANA residues per 100 amino acid residues

flocculate. As shown in Figure 13.5, they only do so when by far the greater part of the κ-casein has been split. Even then, flocculation at 30°C proceeds many times slower than the rate predicted for rapid flocculation. (See Equation 12.8.) Consequently, there still may be some repulsion.

The forces keeping the flocculated paracasein micelles together are probably not exclusively Van der Waals forces. They certainly would be too

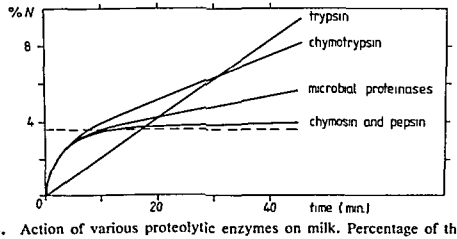



**Figure 13.4.** Action of various proteolytic enzymes on milk. Percentage of the protein *N* becoming soluble after adding the enzyme. The dotted line indicates the *N* in the "caseino-macropeptide." Approximate examples. Largely after C. Alais, *Science du Lait*, 3rd ed., 1974.

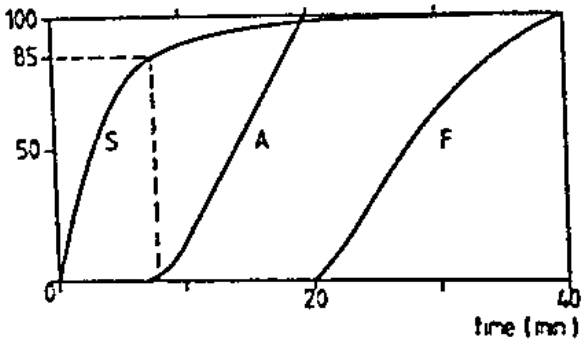



**Figure 13.5.** Renneting of milk. Degree of splitting of κ-casein (S), aggregation of paracasein micelles (A), and firmness (approximately a yield stress) of gel (F) in percentage of their values at 40 min as a function of time after adding rennet. Only meant to illustrate trends. After various sources.

weak for the smaller micelles. There is evidence for an exposed cluster of positively charged groups in para-κ-casein. If the side groups involved are blocked or modified, normal clotting is prevented. This may point to electrostatic interaction between positively and negatively charged regions at the paracasein micelles. It often is assumed that hydrophobic bonding is involved. This cannot be ruled out, but the only evidence is the temperature dependence of clotting, and that can be explained in another way. (See below.) After the micelles are flocculated, they lose their identity over several hours (i.e., they seem to fuse into larger units). Presumably, rearrangement of calcium phosphate throughout the micellar region occurs, and binding between paracasein micelles now may be seen as partly identical to the binding between submicelles in a casein micelle.

Several factors affect the rate of flocculation (see also Figure 13.3):

1. The fraction of κ-casein that is split strongly influences the rate. (See Figure 13.5.) Other conditions being equal, a milk with a higher proportion of κ-casein clots more slowly. But as κ-casein content and micelle size are related, it is difficult to ascertain the primary cause.
2. The temperature dependence is very strong ($Q_{10} \approx 30$ or $Q_1 \approx 1.4$). This should not be interpreted as representing an activation energy (clotting then would be about $10^{-40}$ times as fast as observed). It appears that the free energy of activation for flocculation strongly increases with decreasing temperature. This may be caused by increased repulsion, presumably caused by protruding chains of β-casein, and by decreased Van der Waals attraction, caused by swelling of the micelles.
3. pH as such seems to have little effect, but it does affect other factors, especially items 4 and 5 below.
4. The $Ca^{2+}$ activity is fairly critical. Flocculation usually occurs if it is 1.5–2.0 m*M* or higher (including $Mg^{2+}$). Often, $CaCl_2$ is added to milk to enhance clotting.
5. The lower the content of calcium phosphate of the micelles, the higher the $Ca^{2+}$ activity needed to cause flocculation.

6. Flocculation being a second-order reaction, its rate increases with increasing concentration of casein.

Renneting or clotting time (i.e., the time needed for formation of visible aggregates or for formation of a firm gel) is the resultant of two reactions. The enzymatic reaction is largely determinant, as flocculation time is insignificant compared to splitting time. But this is not always so, particularly at low temperatures. At 4°C, for instance, enzyme action may be complete in about 3 h, while clotting takes a week. This difference in rates allows the reactions to be separated. We may let the enzyme act at, say, 5°C, and after that clotting can be achieved very quickly (within a minute) after rapid warming to, say, 30°C. We also may separate the reactions by first lowering Ca content of the milk, then adding rennet, and adding $CaCl_2$ after the enzyme action is complete.

Under normal conditions, sufficient rennet is added (say, 300 ml containing 4 μmol of chymosin to a cubic meter of milk) to clot the milk in about 30 min. The rule of Storch and Segelcke applies; it states that renneting time $t_r$ is inversely proportional to rennet concentration, $c$. This rule is empirical, not the outcome of some fundamental aspect of enzymatic or other reaction kinetics. Accurate measurements usually show

$$t_r = \frac{K}{c + t_0} \tag{13.1}$$

where $t_0$ is on the order of 20 s and the inverse rate constant $K$ varies, for instance, by a factor of 2 among lots of milk, or rather more between individual cows' milk. Usually there is a clear influence of season. The variation in $t_r$ is smaller when $CaCl_2$ has been added. Equation 13.1 is used commonly to determine $c$ and consequently the strength of a rennet preparation. A standard rennet (preferably pure chymosin) has to be used as a reference because of the variability of $K$.

An additional cause for the variation in renneting time may be mastitis. Most samples of mastitic milk (as defined by a high leukocyte count) clot slowly and yield a weak gel; the explanation is not clear.

### 13.3.4. Gel Formation and Syneresis

The flocculating paracasein micelles first form small aggregates that often have an irregular and elongated shape. The aggregates then form a network with pores of a few micrometers in width. When fat globules are present, part of the pores are wider; these (and possibly other) particles must be accommodated. The fat globules are entrapped in the network and do not form part of it (unless they have some casein in their surface layers). As soon as a three-dimensional network forms throughout the milk, it becomes a gel or, to use a common term, a curd. The gel becomes firmer for a while

(Figure 13.5), and this implies that still more bonds between casein micelles are formed, despite the fact that almost all of the κ-casein has been split.

Even after formation of the gel network, many more bonds between micelles can be formed in principle, because a much more compact packing of the micelles is possible. When this occurs liquid is expelled from the gel, a process called *syneresis*. The formation of new bonds is difficult because the rigidity of the network hinders reactive sites at paracasein micelles from reaching each other; this is purely a geometric or steric hindrance. Probably, other bonds have to break before new ones can form. Consequently, the tendency to syneresis of the curd is small; usually the endogenous syneresis pressure that the contracting curd exerts on the liquid is only on the order of 1 Pa (corresponding to 0.1 mm of water). In practice, syneresis always is enhanced by applying external pressure to the curd or deforming it. In this way, some bonds presumably are broken, and new ones can form more easily. Very small pressure gradients, such as produced by stirring or by cheddaring, can enhance syneresis considerably.

If the gel is constrained geometrically, syneresis is impossible. But then microsyneresis takes place, which means that small regions in the gel shrink while larger pores are formed in adjacent regions. Liquid has to stream through the pores of the gel, and this causes a resistance to syneresis, the larger as the pores are narrower and the transport distance is larger. Therefore, in cheese making the curd usually is cut into pieces. This considerably speeds up syneresis. Still, the center of a piece of curd may show little syneresis while the outer layer shrinks, yielding a lower moisture content, and (in extreme cases) a kind of skin forms. It takes a long time for the moisture distribution to become homogeneous.

Another cause for syneresis is shrinkage of the paracasein micelles. If the building blocks of a network become smaller, the whole network will shrink.

Several factors affect the rate and extent of syneresis (see also Figure 13.3):

1. Size of the curd pieces. Often initial syneresis rate is about proportional to specific surface area of the curd pieces.
2. Pressure gradients, mechanically applied. See above.
3. Calcium phosphate in the micelles. This decreases syneresis, presumably because of the rigidity it imparts to the curd.
4. pH. When lowering the pH, calcium phosphate dissolves and casein conformation becomes more compact; hence syneresis increases. This is a strong effect. But a gel made from milk by acidification to a pH of about 4.6 (without adding rennet) hardly shows syneresis.
5. Addition of $Ca^{2+}$. A little usually increases, but more of it decreases syneresis, the latter presumably as the calcium phosphate content of the micelles increases.
6. Temperature. This has a very strong effect, and below 18°C syneresis

virtually stops This is comparable in cause to the much diminished flocculation rate of paracasein micelles at low temperature

Syneresis (e g , expressed as the amount of whey expelled) is at first about proportional to $t^{0.7}$ where $t$ is time after cutting the curd Later on the rate decreases There is, of course, a lowest possible moisture content At high temperature (~ 35°C) and low pH (~ 5 2) the lowest obtainable ratio of water to paracasein is about 1 2 (if no fat globules are present), which means that the curd has shrunk to about a fifteenth of its original volume If the curd is kept at milk pH, shrinkage to about a third of the volume can be achieved Moisture content after syneresis thus depends primarily on temperature, pH, pressure gradients applied, and fat content Fat globules, acting as passive spacers, hinder further shrinking In practice, syneresis usually is stopped at the desired level by lowering the temperature

Often, we want the shrunken curd grains to fuse into a more or less homogeneous and coherent mass Close contact between curd grains can be achieved by pressing them together But for the actual fusing new bonds between paracasein micelles have to be formed This is possible only if the pH still is decreasing while the grains are pressed together Pressing after the pH has obtained its final value does not produce a coherent mass Adding $CaCl_2$ to the milk promotes curd fusion

### 13.3.5. Effect of Heating

A heat treatment of greater intensity than low pasteurization, thus involving denaturation of whey proteins, causes an increase in rennet clotting time, a weaker curd, and impaired syneresis If the heat treatment is severe (e g , 30 min at 90°C) the milk does not clot at all The clotting time becomes even longer when the heated milk is kept longer and at a lower temperature before adding rennet Addition of $CaCl_2$ can restore the clotting characteristics of the milk to some extent, if the heating was not too severe If no whey proteins are present during heating, rennet action is not affected

The explanation of these observations is probably as follows $\beta$-lactoglobulin is heat denatured and reacts with $\kappa$-casein The $\kappa$ casein thus altered is not (or is less) sensitive to cleavage of the Phe–Met bond by chymosin, this pertains in particular to those $\kappa$ casein molecules which are poor in carbohydrate Consequently, rennet action is incomplete, and the casein micelles remain fairly insensitive to $Ca^{2+}$ On the other hand, the heat-denatured $\beta$-lactoglobulin and most other whey proteins become associated with the casein micelles (This leads to an increase in cheese yield, if the milk still will clot ) Heat-denatured $\beta$-lactoglobulin is very sensitive to $Ca^{2+}$ Moreover, the calcium phosphate content of the micelles increases appreciably because of the heat treatment Consequently, the higher stability caused by the diminished cleavage of $\kappa$ casein is compensated for to some

extent by other factors. The dissolution of the excess calcium phosphate during cold storage (see Section 4.5) diminishes the compensating effect.

## 13.4. AGE GELATION

When sterilized concentrated milk products are kept they often gel, for instance, after one to twenty-four months. Usually, the viscosity of the product remains constant for a long period, or even slightly decreases; then it suddenly rises (age thickening), and soon the milk forms a gel that cannot be redispersed. (See Figure 13.6.) The casein micelles have formed a network, but the explanation is still uncertain. It appears as if two different mechanisms may be responsible.

One is proteolytic breakdown of the casein, making the micelles susceptible to aggregation, somewhat as in renneting. This may happen in UHT milk because proteinases, particularly some bacterial proteinases, may be sufficiently resistant to heat treatment. Keeping the raw milk for some days at a low temperature then may aggravate the problem considerably, because of the growth of psychrotrophic bacteria producing heat-resistant proteinases. However, not every proteinase (or combination of proteinases) causes gelation, and sometimes the casein just dissolves, leaving a clear solution (if no fat is present) and a sediment of calcium phosphate. In extreme cases, gelation occurs within two weeks. Milk proteinase (plasmin), if present, may cause dissolution of casein rather than gelation.

The second, more common mechanism is even less understood. Chemical reactions are essential, which points to chemical cross-linking. The principal reaction(s) appears to be different from that causing heat coagulation; several variables have a very different effect on either instability. Age gelation also occurs in the absence of whey proteins. Blocking of thiol groups does not

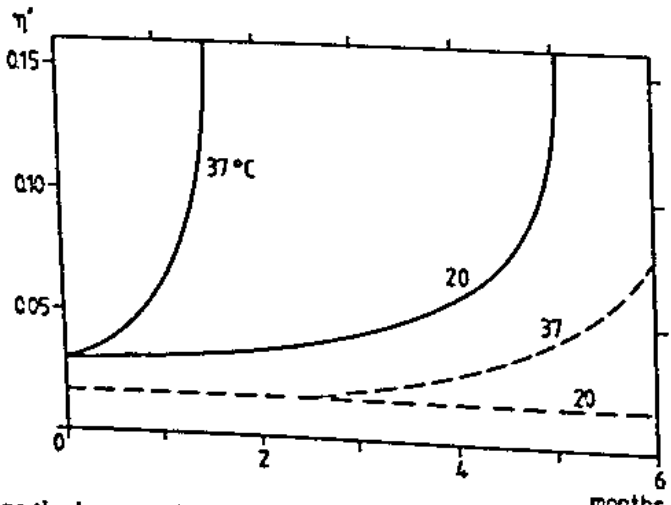



Figure 13.6. Age thickening of concentrated milk. Apparent viscosity ($\eta'$, Pa·s) as a function of time of storage at two temperatures (parameter). The broken lines concern milk to which polyphosphate had been added. Approximate results from various sources.

prevent gelation; nevertheless, it is fairly certain that redox reactions are involved. Maillard reactions have been held responsible, both for causing and for delaying gelation. Probably, several reactions are involved, and under different conditions or for different products the mechanism responsible may well be (partly) different.

Age gelation typically occurs in highly concentrated products at high temperature and takes a long time. A similar phenomenon occurs during storage of milk powder; its protein becomes insoluble slowly. The following factors seem to affect gelation, but it is unknown what mechanism was responsible during the experiments performed to estimate the effects; so there may be exceptions:

1. Differences occur among lots of milk, often with a marked effect of season.
2. Degree of concentration influences gelation. Plain sterilized milk hardly ever gels, while milk concentrated to a third of its volume nearly always does after long storage. Obviously, there is an optimal concentration for gelation; in milk powder, the higher its water content, the sooner its protein becomes insoluble. This parallels the development of Maillard products.
3. Increasing temperature generally accelerates gelation. (See Figure 13.6.) A $Q_{10}$ of about 4 sometimes has been found, but it may be quite variable, and values less than 1 have even been reported (i.e., quicker gelation at lower temperature).
4. Intense heat treatment delays gelation. Consequently, it is typically a problem in UHT products rather than in (concentrated) milk sterilized in bottles or cans. Preheating the milk before concentrating it in the manufacture of evaporated milk has no effect, but heating the concentrate to temperatures above 110°C for several minutes improves the stability to gelation.
5. Cold storage of evaporated milk, after concentrating but before sterilizing, considerably accelerates gelation, also if no bacterial proteinases are formed. Perhaps, $O_2$ uptake is involved.
6. Addition of sugar delays gelation, particularly when added after concentrating. The explanation is unknown, but it allows the manufacture of sweetened condensed milk of good keeping quality without applying intense heat treatment.
7. Addition of polyphosphates (e.g., 2–3 g sodium hexametaphosphate per 100 g casein) considerably delays gelation. (See Figure 13.6.) Such phosphates bind to protein, particularly to $\kappa$-casein. Addition of orthophosphate has no or an adverse effect. Consequently, polyphosphates should be added after sterilization; they are hydrolyzed to yield orthophosphate at high temperatures. The addition of $CaCl_2$ or the alteration of pH usually has little and often nonconsistent effects.
8. Addition of $MnSO_4$, $ZnSO_4$, or $FeSO_4$ usually delays gelation.

Homogenized UHT cream also is susceptible to age thickening. Now the casein-covered fat globules participate in forming at first aggregates (thereby appreciably increasing viscosity) and eventually a gel. Besides the variables mentioned, those affecting the amount of casein associated with the fat globules influence the rate of gelation. (See Section 14.4.) Addition of Ca-sequestering agents or sweet-cream buttermilk before homogenization thus causes delay of age thickening.

The slow insolubilization of the protein in frozen milk (Section 17.4) may be similar to age gelation, though it is more likely to be a salting-out effect.

## 13.5. HEAT STABILITY

When milk is held at temperatures above the boiling point it eventually coagulates, the higher the temperature the sooner. Few subjects in dairy chemistry have been studied more intensively and are understood less. Nevertheless, important practical results have been obtained, and these will be stressed in this section. It is by no means an endeavor to review exhaustively the multitude of observations and hypotheses on heat stability.

### 13.5.1. Phenomena Involved

It is the casein that undergoes heat coagulation. The cause is not heat denaturation as suffered by globular proteins. Casein is not denaturable, and the temperature dependence of the heat coagulation of milk is much weaker than that of the heat denaturation of proteins. (See Section 10.2.) The casein micelles aggregate, and this is not merely a flocculation caused by a lack of colloidal stability of the micelles at the prevailing temperature, because the aggregates do not redisperse after cooling again. The composition of the milk serum is changed considerably because of the heating (Section 10.1), but restoring the original environment does not dissolve the aggregates. Even addition of agents that break H bonds, reduce –S–S– linkages, or dissolve calcium phosphate leaves the aggregates intact. Consequently, chemical change of the casein must have occurred. It is known that casein suffers dephosphorization, partial hydrolysis, and several cross-linking reactions at high temperatures. (See Section 10.3.)

Heat stability usually is defined as the time needed to cause visible coagulation of milk at a given temperature. The unresolved question is what reaction determines the rate of coagulation. Perhaps some chemical reaction renders the micelles unstable, after which they aggregate as soon as they meet, regardless of conditions such as temperature (as with the heat-induced change of β-lactoglobulin that enables it to react with κ-casein). In that case the rate of the chemical reaction(s) would be determinant. Perhaps the casein micelles have to come close together before any cross-linking reaction between them can occur. In that case the interaction energy between the micelles, hence colloidal aspects, may be rate determinant. (See Sections

12 2 and 12 3 ) It appears that colloidal interactions come into play, though they are not the ultimate cause of the coagulation

Figure 13 7 shows examples of the dependence of coagulation time on pH, and it suggests that at different pH different reactions are rate determining It also follows that the pH dependence can differ among individual milks Most cows give milk of type A, some give type B, mixed milk is generally of type A But the shape of curve can show more variation than indicated in Figure 13 7 The pH optimum (if present) can vary from 6 6 to 6 9 among lots of milk Consequently, the pH of the milk itself can be below, at, or above the optimum To compare the heat stability of lots of milk, we therefore should determine it at a range of pH values, including that giving *the maximum stability*

The pH as given in Figure 13 7 is at room temperature before heating At high temperature the pH will be much lower, but this is mainly a result of the increased dissociation of water, and a lower pH does not imply a more acid environment *(See Section 11 3 ) The precipitation of amorphous tri*calcium phosphate at high temperature, thereby liberating protons, causes a further decrease in pH Furthermore, acid is produced during heating, and the pH (as measured at room temperature) at the time of coagulation will be much lowered, the more so as the time needed for coagulation is longer and the temperature higher The rate of change of pH also depends on the initial pH (Figure 10 1) and on the availability of $O_2$, because this is needed for the formation of acids from lactose The rate of acid production during heating is certainly the most important factor in the heat coagulation of (unconcentrated) milk The pH at coagulation is always low (< 6 2, as measured at room temperature) Keeping the pH at its original value during heating by adding alkali prevents heat coagulation, even after several hours

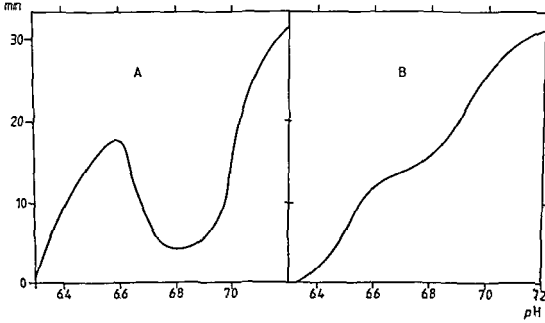



**Figure 13 7.** Examples of the heat coagulation time (minutes at 140°C) of milk as a function of pH

of heating. The apparent activation energy for heat coagulation is usually equal to that for acid production.

A solution of Na-caseinate is very heat stable (more than 1 h at 140°C and pH 6.7) though it eventually coagulates. A dispersion of casein micelles in a milk–salt solution is far less stable; the heat stability shows a pH dependence like type B. (See Figure 13.7.) Adding lactose to the dispersion decreases heat stability over the full pH range, undoubtedly because more acid is formed during heating. It appears that the colloidal stability of the micelles is important. The lower the pH and the higher the $Ca^{2+}$ activity, the lower the stability. Also, increasing average size, decreasing voluminosity, and increasing content of calcium phosphate of the micelles decrease their heat stability. At high temperatures, the amount of calcium phosphate associated with the micelles increases. (See Section 4.5.) The role of colloidal phosphate is, however, different at higher pH (roughly > 7); it now increases the heat stability, presumably by its buffering action.

If β-lactoglobulin or whey proteins are added to a suspension of casein micelles in protein-depleted milk serum, the heat stability curve usually is converted from type B into type A. At low pH (roughly < 6.7) heat stability is increased, and this may be caused by the β-lactoglobulin diminishing the rate of decrease of the pH. At higher pH coagulation time is remarkably decreased, causing a minimum in the curve. Reactions of thiol groups are involved; addition of a blocking agent (like N-ethyl maleimide) before heating largely eliminates the effect of β-lactoglobulin. Most probably, it concerns the reaction between β-lactoglobulin and κ-casein, because addition of an excess of κ-casein reconverts the type A curve into type B. But other factors also play a part. If the content of colloidal calcium phosphate and/or the $Ca^{2+}$ activity are very low, there is no minimum, despite the presence of sufficient β-lactoglobulin. Heat-denatured β-lactoglobulin is very sensitive to $Ca^{2+}$ ions, and particularly if the latter are present during heating, a $Ca^{2+}$ activity as in milk would suffice to make this protein insoluble. Since the denatured β-lactoglobulin becomes associated with the casein micelles, this may play a part in the heat stability. In the range near the minimum of the (type A) curve coagulation occurs in two steps, and the first rapid step predominantly involves the larger casein micelles, while the coagulum contains a high proportion of calcium phosphate. Other whey proteins that are sensitive to $Ca^{2+}$ ions after heating (e.g., α-lactalbumin) have more or less the same effect as β-lactoglobulin. These proteins also can bind calcium phosphate on heating.

### 13.5.2. Important Variables

Numerous factors affect the heat stability, and some follow from the discussion in the preceding section, notably pH. Unless stated otherwise, we will compare the stability at the optimal pH. The magnitude and even the sign of the effect of some variables may depend on other variables.

**Test Conditions.** Heat coagulation times decrease with temperature. (See Figure 13.8.) On average, $Q_{10}$ is about 3, apparent activation energy is about 150 kJ·mol$^{-1}$ throughout most of the pH range, though $Q_{10}$ tends to increase somewhat at higher pH. Among lots of milk $Q_{10}$ may vary considerably (e.g., from 2–7).

At pH above 6.7, heat stability (of unconcentrated milk) tends to decrease considerably with available $O_2$. Hence, a larger headspace volume and rocking of sample tubes during heating may decrease coagulation time, for instance by a factor of 3 at a pH near 7. This must be caused by the increased rate of acid production.

**Composition.** The wide variability in heat stability among lots of milk must be caused by differences in composition. There is usually a large effect of season (coagulation time may vary by a factor of 4), presumably for the greater part caused by stage of lactation. The variation between individual cows also is considerable.

Part of the variability follows from factors involved in colloidal stability: $Ca^{2+}$ activity, casein micelle size, phosphate content of micelles, and their voluminosity. Variation in protein composition appears to be too small to have much effect on heat stability. The most important component causing variation mostly is urea. Average coagulation time may be 14 min at 140°C for milk with 0.25 g of urea per kilogram of milk, and 26 min for 0.5 g·kg$^{-1}$; such variations are well within the normal range of urea contents. The action of urea is partly through its slight inhibition of acid formation. At higher temperature urea is converted partly into ammonium cyanate, and this compound can block $\epsilon$-amino groups and affect thiol groups of the proteins. A watertight explanation has not been given. Urea content has no effect on coagulation near the minimum in the pH curve.

**Concentration.** The heat stability of concentrated milk is considerably less than that of milk. (See Figure 13.8.) Highly concentrated (skim) milk (e.g., > 40% s.n.f.) gives a firm gel on heating, even at temperatures near

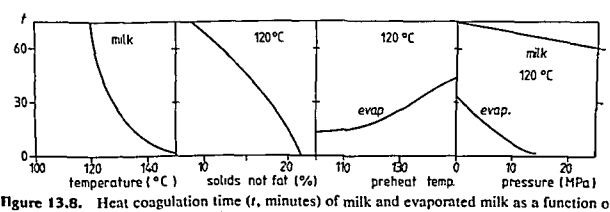



**Figure 13.8.** Heat coagulation time ($t$, minutes) of milk and evaporated milk as a function of test temperature, content of solids-not-fat of the milk, preheat temperature (duration, e.g., 4 min), and homogenization pressure. Approximate results from various sources.

100°C. There is little correlation between the heat stability of fresh milk and that of the concentrated milk prepared from it. Urea content, for instance, generally has no influence on the heat stability of concentrated milk. The coagulation mechanism is probably different, at least over much of the pH range. The difference in heat stability is especially striking at pH above 7; here, the stability of evaporated milk is very low (mostly < 1 min at 130°C), while most unconcentrated milk is very heat stable. (See Figure 13.7.) This implies that the decrease in pH during heating up to the coagulation point (which is considerable in unconcentrated milk) is insignificant in concentrated milk, which also points to a different coagulation mechanism. Conceivably, heat coagulation of concentrated milk at not too low pH is similar to age gelation. The concentration as such causes the pH to decrease and the pH optimum also shifts to a somewhat lower value; there is no minimum in coagulation time. Figure 13.8 gives the coagulation time at the pH optimum. Milk concentrated by ultrafiltration (rather than by evaporation) is much more heat stable than evaporated milk.

**Preheating.** For unconcentrated milk, preheating mainly causes a shift of the heat coagulation curve to lower pH values, and the stability at the pH optimum is hardly affected. But a very intensive preheat treatment (e.g., a few minutes at 150°C) causes heat stability to increase over the whole pH range, and the minimum in the curve disappears. Perhaps the second heat denaturation of β-lactoglobulin, which occurs near 140°C, is involved.

Concentrated (evaporated) milk hardly can be sterilized without preheating the milk before concentrating. The heat-stability maximum is shifted to lower pH values and becomes higher. (See Figure 13.8.) The pH of the milk before preheating also affects the heat stability of the evaporated milk, its optimum being, for instance, 6.45. The beneficial effect of preheating must be caused at least partly by reactions of the β-lactoglobulin; addition of this protein to the milk decreases the heat stability of evaporated milk, but this decrease can be eliminated largely by proper preheating.

**Additives.** Several additives (some of which are illegal) may enhance heat stability. NaOH or sometimes HCl is used to adjust the pH. $Na_2HPO_4$ also can be used for this purpose, and it usually gives an even better heat stability, presumably because it lowers $Ca^{2+}$ activity.

Agents that interfere with free thiol groups can be beneficial. The effect of N-ethyl maleimide already has been discussed. Addition of 0.05% $H_2O_2$ to milk before evaporation considerably enhances the stability of the concentrated milk; higher additions become detrimental. Adding 0.5–1.0 mg $Cu^{2+}$ per kilogram of milk, after preheating and before evaporation, usually gives a marked increase in heat stability of evaporated milk.

As will be clear from the discussion of composition above, addition of urea can increase the heat stability of (unconcentrated) milk. Several aldehydes in about 20 m*M* concentration have a similar effect. Those aldehydes

that are effective are like urea in that they diminish the rate of acid production during heating.

**Homogenization.** This process is discussed in Section 14.4. Suffice it here to say that it causes a considerable transfer of milk protein, predominantly micellar casein, from the plasma to the newly formed fat globules. This phenomenon must be involved in the detrimental effect of homogenization on heat stability. Homogenization of skim milk makes no difference, and the heat stability of fat-containing products decreases with increasing fat content and increasing homogenization pressure, both of which go along with an increase in the amount of protein transferred. In the case of cream, this seems to be the main factor in determining heat stability. Examples are in Figure 14.10. It shows that higher homogenization temperatures, up to about 70°C, lead to a lower heat stability. Still higher temperatures or preheating of the cream drastically reduces coagulation time.

Homogenization has little effect on milk, but it renders concentrated milk far less heat stable. (See Figure 13.8.) The detrimental effect can be offset for a considerable part by preheating the milk before concentration. The effect of homogenization temperature seems to differ from that in cream.

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# 14

# Milk Fat Globules

Nearly all of the fat in milk is in separate small globules, some 0.1–15.0 $\mu$m in size. Milk is thus an oil-in-water emulsion, and this fact has several consequences for its properties. No emulsion is entirely stable. Any physical or chemical reactions in the fat must start separately in each globule. All interactions between fat and plasma must occur across the interface between the phases. These observations apply to most milk products as well.

Some physical properties, such as color and viscosity of milk and its products, are influenced particularly by the dispersed state of the fat. Other properties, such as refractive index, osmotic pressure, and freezing point, are not influenced significantly by the fat because it is not dissolved. Electric conductance and dielectric constant are affected somewhat by the fat globules, but the predominant variable is the aqueous phase. Most effects mentioned, and others as well, depend on fat content and its composition. Several also depend on the size distribution of the fat globules.

## 14.1. PROPERTIES

### 14.1.1. Size Distribution

General aspects of size distributions are discussed in Section 12.4. An example of the number and volume frequency distributions of milk fat globules is in Figure 14.1. It is seen that milk contains very many small globules, which comprise only a small fraction of the total fat. The total globule number is about $15 \times 10^9 \cdot ml^{-1}$ of which about 75% are less than 1 $\mu$m in diameter. The number average diameter, $d_{10}$, is therefore small, on average about 0.8 $\mu$m. But the large number of small globules is difficult to determine, and this implies that estimates of $d_{10}$ usually are not accurate. We prefer to use the volume surface average diameter $d_{vs}$; it is on average about 3.4 $\mu$m for

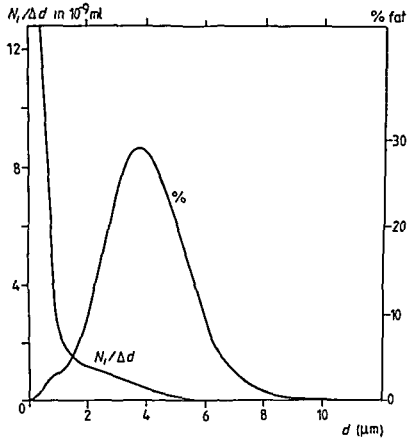



**Figure 14 1.** Average size frequency distribution of the fat globules in milk of a Friesian cow ($d_{vs}$ = 3 4 µm fat content 3 9%) Number ($N/\Delta d$) and volume frequency (% of the fat) are given per micrometer class width After P Walstra *Neth Milk Dairy J* 23 (1969) 99

milk of several breeds of cow The $d_{vs}$ relates total fat volume to its total surface area, and this is an important parameter, for instance, in studies of the fat globule membrane Fat surface area mostly ranges between 1 2 and 2 5 $m^2\ g^{-1}$ of fat

Besides average size, the width of the size distribution should be considered, we use the surface-weighted relative width $\sigma_s$, defined in equation 12 15 For milk from one cow $\sigma_s$ equals about 0 4, it varies little among cows The shape of the volume frequency distribution is almost constant, too, among individual cows' samples, though average diameter and total amount of fat vary widely. This implies that the different size distributions can be made to coincide merely by altering the scales In mixed milk, the distribution naturally is somewhat wider, $\sigma_s$ of about 0 45

Figure 14 2 shows that the size distribution of milk fat globules has some peculiarities Apparently the distribution can be split into three subdistributions

1. Small globule, comprising most of the globules (e g , 80%) but only a small percentage of the fat
2. Main, comprising most (e g , 95%) of the fat, this is the one of constant shape
3. Large globule, a small tail of very large ones

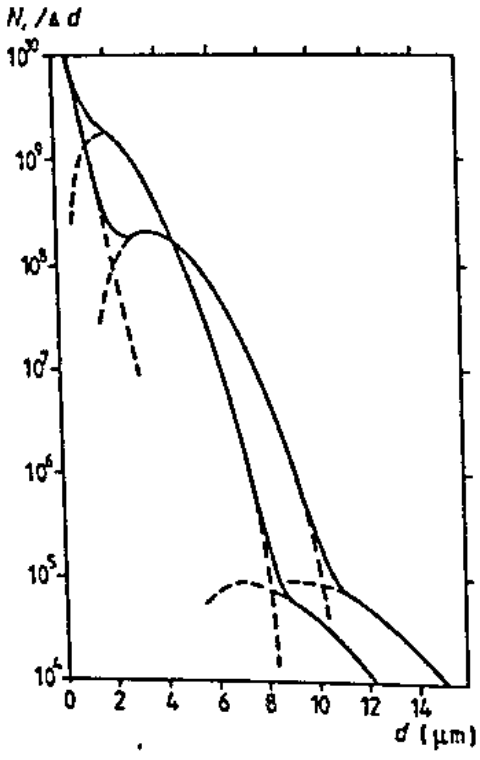



**Figure 14.2.** Number frequency ($N_i/\Delta d$ per milliliter per micrometer class width, logarithmic scale) versus diameter of fat globules in a milk with small and one with large fat globules. The dotted lines indicate a division into subdistributions. After P. Walstra, *Neth. Milk Dairy J.* 23 (1969):99.

Figure 14.2 also shows that the number frequency sometimes (e.g., in early lactation) may show a secondary minimum near 3 $\mu$m. The cause is that the small globule subdistribution is almost constant while the main subdistribution may vary in average and quantity.

Average globule size varies among breeds, among individual cows, and with stage of lactation. (See Figure 14.3.) The size distribution can be altered greatly by treatment, particularly by homogenization. (See Section 14.4.) Examples of size parameters are given in Table 14.1, but fairly large deviations from the quoted values may occur.

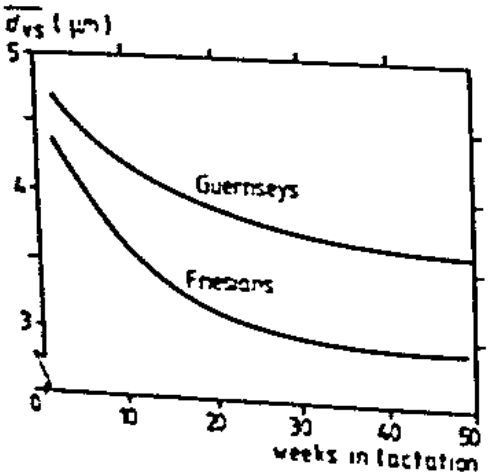



**Figure 14.3.** Average globule size (average $d_{vs}$ values) from several cows against stage of lactation for Guernsey and Holstein–Friesian cows. Recalculated from M. H. Campbell, *Bull. Vt. Agric. Exp Sta.* 341 (1932).

**Table 14.1. Approximate Examples of Some Parameters of the Fat Globule Size Distribution of Milk and Milk Products**

| Milk Product | % fat w/w | $d_{vs}$ ($\mu$m) | $\sigma_s$ | $A$ ($\mu m^{-1}$)[a] | mean free distance[b] ($\mu$m) | mean free distance[b] $z/d_{vs}$ |
|---|---|---|---|---|---|---|
| Whole milk, Friesians | 3 9 | 3 4 | 0 45 | 0 077 | 12 2 | 3 6 |
| Whole milk, Jerseys | 5 2 | 4 5 | 0 45 | 0 077 | 12 0 | 2 7 |
| Homogenized milk | 3 5 | 0 6 | 0 85 | 0 380 | 2 3 | 4 1 |
| UHT milk | 10 | 0 3 | 0 85 | 0 790 | 1 2 | 4 1 |
| Light cream | 10 | 3 5 | 0 45 | 0 190 | 4 5 | 1 3 |
| Homogenized cream | 18 | 1 0 | 0 95 | 1 200 | 0 6 | 0 6 |
| Whipping cream | 35 | 4 0 | 0 50 | 0 590 | 0 7 | 0 2 |
| Evaporated milk | 8 | 0 4 | 0 85 | 1 300 | 0 6 | 1 6 |
| Sweetened condensed milk | 8 | 1 0 | 0 90 | 0 530 | 1 1 | 1 1 |
| Ice-cream mix | 10 | 0 6 | 0 90 | 1 100 | 0 7 | 1 2 |
| Separated milk | 0 04[c] | 1 3 | 0 50 | 0 002 | 500 0 | 380 0 |

*Note* From various sources

[a] $A$ = surface area per unit volume of product 1 $\mu m^{-1}$ = 1 $\mu m^2$ $\mu m^{-3}$ = 1 $m^2$ $ml^{-1}$

[b] Equation 12 18

[c] Globular fat only

### 14.1.2. Composition

Fat composition is discussed in Sections 5 1 and 5 2 It varies among cows, so the fat globules in mixed milk differ in composition Mixing the milk gives the serum components a homogeneous distribution but obviously does not do so for the fat Even in the milk from one milking of one cow, fat globules differ much in composition, judging from their refractive index $n^D$ The spread (standard deviation) in $n^D$ among globules is about $7 \times 10^{-4}$, and the spread of $n^D$ of milk fat among cows at any one time is often about the same Compare this with the range of about $15 \times 10^{-4}$ in $n^D$ for average milk throughout the year It follows that in an average milk about a fourth of the fat is in globules having a very firm (high-melting) fat and a fourth is in globules with a very soft (low-melting) fat

It often has been assumed that fat composition is correlated with globule size, but the only clearcut case of such a relation stems from the fact that small globules have a greater amount of membrane lipids and that the latter differ greatly in composition from the core lipids (See Table 5 1 ) The globules have on average about 4 mg of membrane lipids per square meter of

surface area (0.8% of the fat), and thus globules of 0.4, 2.0, and 10.0 $\mu$m diameter contain 6.50%, 1.30%, and 0.26% of membrane lipids, respectively.

### 14.1.3. Fat Crystallization

Crystallization of milk fat is discussed in Section 5.7. We will restrict ourselves here to some aspects of crystallization of globular fat. The main differences between fat crystallization in globules as compared to that in bulk are:

1. Supercooling can be much deeper because of the limited number of catalytic impurities for nucleation. (See Figure 5.5.) Nucleation at the globule boundary (at the inside of the membrane) does not occur normally, at least not above 4°C.
2. Crystallization often is isothermal because heat can be removed easily from milk or cream and not so easily from a crystallizing bulk fat. This causes differences in polymorphs and in compound crystals.
3. Crystal size is much smaller in globules, partly for the trivial reason that crystals must remain less than $d$. But crystals in globules are often much smaller than $d$ (invisible in a light microscope), for reasons not fully understood.

According to Section 12.1.4, crystals should be held at the oil–water interface (i.e., the fat globule boundary) because this gives the lowest surface free energy. However, this usually does not happen. The crystals attract each other by Van der Waals forces, and as there is no repulsion between them (except at atomic distances) they flocculate and do so very quickly. They are anisometric so they easily form a network in which the liquid fat is held. This prevents them from reaching the OW interface. But when the globule is deformed (as happens during churning), crystals may become attached to the interface, forming a tangential layer; more crystals usually arrange themselves parallel to this outermost layer. Sometimes such an arrangement also forms spontaneously, for unknown reasons. This alignment can be observed easily in a polarization microscope with crossed nicols. The globules then show a birefringent outer layer. It must be assumed that at least some of the crystals then partly stick out of the globules.

It should be noted that in freeze-etch electron micrographs concentric layers of apparently crystalline fat can be seen at the boundary of some globules, but this may be an artifact because there is no other evidence for such a configuration.

## 14.2. EMULSION STABILITY

Milk, cream, and several milk products are emulsions. In many cases physical stability is desired; the emulsion should not change on standing. How-

ever, change in the emulsion is desired in processes such as separation of cream from skim milk, aggregation of globules in the whipping of cream and the freezing of ice cream, and breaking of the emulsion in churning.

### 14.2.1. Types of Instability

Various types of physical instability can occur, as illustrated in Figure 14.4. Often, these changes cannot be differentiated by simple visual observation but, for instance, by microscopy. It should be realized that the various changes may occur simultaneously. Some changes (e.g., creaming) always

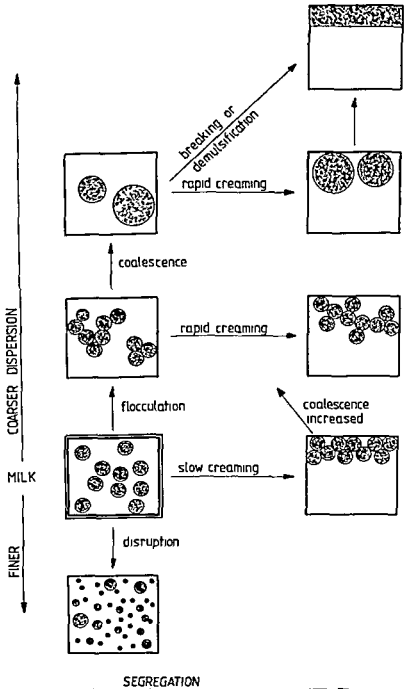



**Figure 14.4.** Different types of emulsion instability. Simplified and highly schematic. Fat is gray. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

occur, though possibly very slowly. Flocculation and coalescence have an activation energy; thus, they may be retarded or even prevented unless energy is applied to accelerate them.

The situation is more complicated than given in Figure 14.4 because different types of aggregates may occur, as illustrated in Figure 14.5. We have given these types arbitrary names because there is no generally accepted terminology. The aggregates, of course, may be composed of numerous globules.

In *floccules*, the globules keep their identity and do not actually touch; attractive forces between the globules are fairly weak, and vigorous stirring usually suffices to disrupt the floccules. Flocculation does not happen normally with natural milk fat globules, as is discussed in Sections 12.2.3 and 12.2.4. It appears that some of the glycoproteins in the membrane are responsible for most of the (steric) repulsion between globules; enzymatic splitting of these glycoproteins causes the globules to flocculate. Flocculation of fat globules may occur in the cold by means of agglutinin. (See Section 14.3.)

In *clusters*, the globules are held together more strongly. We may envision that the surface layers are fused, or that two globules share a certain amount of surface material. Clustering does not happen where the globules have their natural membrane, but it may occur if they are covered with plasma proteins, as caused by homogenization or other processes leading to fat globule damage. Clusters usually can not be disrupted by stirring, but they are dispersed into globules when dissolving the protein at the globule surface (e.g., by chelating Ca, raising the pH, or adding sodium dodecyl sulfate). Clustering may occur in heat coagulation (Section 13.5) and during homogenization (Section 14.4).

In *granules*, fat touches fat. Granules always contain fat crystals. If the fat were fully liquid, a granule would immediately coalesce further into a larger globule, because of the Laplace pressure (equation 12.2); and this is what happens when a granule is warmed to above the final melting point of the fat. Liquid fat is needed as a sticking agent. We also may envision a granule as a collection of globules held together by "necks" of liquid fat between them. The energy needed to disrupt the granules is very large (mostly at least $10^6\ kT$ for a doublet), and it would take very vigorous stirring with a velocity gradient of some $10^7\ s^{-1}$ to disrupt them.

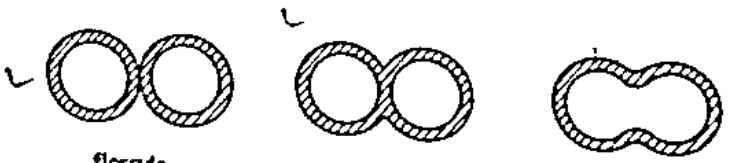



**Figure 14.5.** Different types of aggregates of fat globules. Highly schematic and not to scale. The membrane or surface layer is hatched.

vigorous Liquid motion can promote coalescence The shearing action of the flow apparently may bring two globules that encounter each other closer and longer together, thus facilitating coalescence Moreover, the rate of encounters increases with the velocity gradient or shear rate Still, only a very small proportion, say $10^{-6}$, of the encounters would lead to coalescence Figure 14 6A gives some idea of the effect of shear rate

*Fat content* has little effect on the disruption but particularly affects coalescence (See Figure 14 6B ) This may become obvious when looking at Figure 14 7 The smaller the mean free distance $z$, the higher the probability of two globules meeting each other If $z$ becomes very small the globules cannot avoid each other when the liquid flows, and this further enhances coalescence Finally, at very high fat content, the globules inevitably are pressed together Agitation of 80%-fat cream usually causes a phase inversion if part of the fat is solid, and a water in oil emulsion (almost butter) is obtained

In a cream layer, globules are pressed together somewhat by gravitational forces, and this enhances coalescence When the fat starts crystallizing in the cream layer, this may promote partial coalescence strongly It leads to a fairly solid cream "plug," and in extreme cases a bottle of milk may be inverted without the milk flowing out

Fat content also increases when the water is removed (e g , by evaporation) Heating cream until all of the water is evaporated, leads to breaking the emulsion This process is applied in the production of ghee During drying of milk products some coalescence also occurs

*Particle size* also has an effect Figure 14 6C illustrates the effect of initial globule size on coalescence rate Very small globules are very stable Consequently, in a polydisperse emulsion like milk, the larger globules are especially prone to coalescence They give still larger particles, which coalesce still faster, and the process may thus enhance itself, visible granules often are formed suddenly This applies if the globules are partially solid

If the globules are fully liquid and the milk is agitated, the large ones

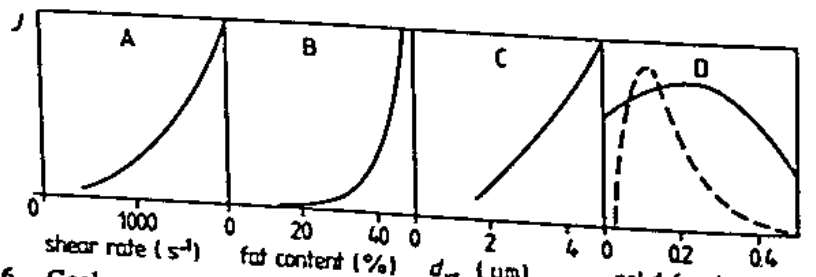



**figure 14 6** Coalescence rate ($J$ number of events per unit time and volume) of milk fat globules in simple shear flow as a function of shear rate fat content average globule size and fraction of the fat being solid Approximate and only meant to illustrate trends The broken line gives the rate of formation of visible granules rather than the coalescence rate under conditions as in churning Mainly after H Mulder and P Walstra *The Milk Fat Globule* (Wageningen Pudoc 1974)

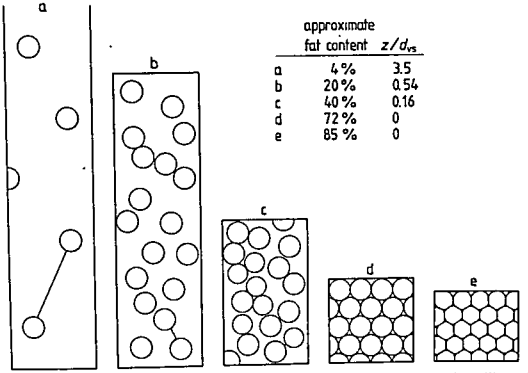



**Figure 14.7.** Illustration of the mean free distance between fat globules in milk and cream. The line segments indicate $z$, Equation 12.18. Remember that a true representation cannot be given in a two-dimensional diagram. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

formed by coalescence eventually will be disrupted again, because the Laplace pressure ($4\gamma/d$) decreases with increasing size and the shearing stress ($\eta G$) remains the same. Consequently, for a certain shear rate there is a maximum globule size that cannot be exceeded. This is illustrated in Figure 14.6D, which shows at zero solid fat content a fairly high (initial) rate of coalescence (solid line) but no formation of visible (i.e., large) granules (broken line).

The *membrane or surface layer* of the globules largely determines the repulsion between them and thus the ease of coalescence; it has other effects on coalescence stability that are less well understood. Natural fat globules are rather stable, though there are differences among lots of milk and though shear and creaming may induce some coalescence, even at blood temperature. In recombined milk, the fat globules have a surface layer of plasma proteins, and this imparts a very good stability under most conditions. But if the surface layer is mixed, part being natural membrane, part plasma protein, as occurs after homogenization, the globules are much less stable. (In homogenized milk, though, globules are too small to coalesce readily.) During handling of milk, fat globules are damaged easily, particularly by contact with air, and this produces mixed membranes to some extent. Consequently, the globules become less stable to coalescence.

If the milk contains surfactants that can replace part of the membrane or proteinaceous surface layer, stability is even further impaired. Such surfactants commonly are included in ice cream. The globules should aggregate

during the freezing and beating process, but owing to previous homogeni zation they would be too small to do so by coalescence Addition of sur factants (preferably before homogenization) makes partial coalescence possible

Still stronger effects can be obtained by adding large quantities (e g , 3%) of some surfactants to milk or cream Heating then suffices for the emulsion to break This is applied to collect most of the fat from a sample of milk, for example to determine fat acidity

*Temperature* affects many of the variables mentioned Generally, at high temperature both rate of coalescence and ease of disruption increase This may give alterations in globule size distribution during pasteurization and sterilization The liquid motion is important, too, of course, so the result much depends on the type of equipment

Cooling to low temperatures seems to diminish irreversibly the protective function of the natural membrane This is particularly clear when the temperature is fluctuated Cooling cream to 5°C (e g , 2 h) then warming to 30°C and cooling it again to 5°C produces a strong increase in its viscosity, and it may even gel This process is called rebodying, and it is caused by a limited degree of partial coalescence induced by the temperature cycling (on warming to 40°C several globules now coalesce)

*Freezing and subsequent thawing* cause considerable coalescence, particularly in cream It is caused primarily by the fat globules being pressed together by the growing ice crystals

Finally, the damage done to fat globules is often expressed in a content of free fat, somehow determined by extraction or centrifugation As stated before (Section 12 1 2) uncovered fat as such cannot exist in milk for longer than 0 1 s The meaning of the test results therefore is highly questionable

## 14.3. COLD AGGLUTINATION

As discussed in Section 6 4, milk contains immunoglobulins, one kind, IgM, acts as an agglutinin This means that IgM can be an antibody to particulate antigens (e g , bacteria) by flocculating them IgM are large molecules, molecular weight about 900 000, maximum diameter about 30 nm

Cryoglobulins are complexes of immunoglobulins (predominantly IgM) and possibly other proteins. They are less soluble below 37°C and precipitate to an increasing extent the more the temperature is lowered Cold agglutination does not involve specific antigen–antibody binding, any particles present can be coprecipitated The optimal concentration of monovalent cations for agglutination is about 0 04 *M* The agglutination is reversed by rewarming

The fat globules in milk are agglutinated upon cooling, and this is the cause of the often observed rapid creaming of fresh cold milk We may envision the following events

1. After cooling the cryoglobulins precipitate onto the fat globules, covering a small but significant proportion of the globule surface

Judging from the time lag (e.g., 30 min) before the flocculation (see below) takes off, the precipitation proceeds much more slowly than would be expected from Brownian motion (at a rate about $10^{-3}$ times that predicted by equation 11.8).

2. The globules have thus become "sticky," and they flocculate at a rate in agreement with equation 11.8. Because cryoglobulin is so large and because only a small part of the globule surface is covered, the repulsion between the globules will not hinder their flocculation appreciably, as they need not come nearer than some 30 nm.
3. The floccules become fairly large, which causes them to rise very rapidly.
4. Large floccules overtake smaller ones and single globules, sweeping those out of the plasma, thereby enlarging themselves and rising still faster.

In this way, a cream layer forms rapidly (e.g., in 1 h) in cooled milk, even in a deep vessel. The floccules are loosely packed, the globules occupying 25–50% of the volume, and the cream layer may contain only 20% fat.

However, the actual mechanism of creaming probably is more complicated than the series of events outlined above. They do not explain the time lag in the initiation of rapid flocculation. Furthermore, the amount of cryoglobulin in milk is itself insufficient to cause flocculation. There is evidence that its effect is enhanced by a lipoprotein that is present. The lipoprotein alone does not cause flocculation, but it interacts with cryoglobulin so that a much lower concentration of the latter is required for cold agglutination. We call the active principle, consisting of lipoprotein and cryoglobulin and possibly other components, agglutinin.

The agglutinin content of milk varies among cows; its concentration is high in colostrum and decreases with advancing lactation. Mixed milk usually contains it, but the amount is not always sufficient to produce appreciable flocculation. The milks of buffaloes, sheep, and goats do not contain agglutinin.

Agglutinin is inactivated by heat. (See Figure 10.4.) Homogenization or even prolonged vigorous stirring of milk (or skim milk) also inactivates it. Homogenized fat globules flocculate in the cold if sufficient agglutinin is present in relation to the enlarged surface area of the fat globules.

Stirring milk at low temperature impairs agglutination because the agglutinin becomes associated with individual fat globules. Warming such milk to 40°C dissociates agglutinin from the globules and largely restores the ability to flocculate when the milk is cooled again.

Agglutinin is found in the cream when milk is separated cold and in the skim milk when it is separated warm. Cold agglutination of fat globules concentrates bacteria in the cream. This is partly nonspecific flocculation, but some streptococci are bound specifically to the fat globules. The agglutinin evidently contains specific antibodies for these bacteria and precipitates like any cryoglobulin onto the fat globules.

## 14.4. HOMOGENIZATION

The purpose of homogenization usually is not to make, but to keep a product homogeneous The main effect is the disruption of fat globules into smaller ones (Figure 14 8), which slows down creaming But it also imparts other effects to the globules Their tendency to disruption or coalescence during handling is much diminished (Section 14 2 2), aggregation of fat globules (not involving coalescence) may occur on heating or souring (Section 14 4 2), and deeper supercooling is needed to induce crystallization (Section 5 7) Moreover, some side effects occur (See Section 14 4 4 )

### 14.4.1. Disruption of Fat Globules

In a homogenizer of the common type, milk is brought under a high pressure ($P$) and forced through a very narrow opening, usually a slit in a spring loaded valve The liquid attains a high velocity ($v$) and

$$v_{max} \approx \left(\frac{2P}{\rho}\right)^{1/2} \qquad (14\ 1)$$

where $\rho$ is liquid density The high velocity (e g , 200 m s$^{-1}$ for $P$ = 20 MPa) gives the liquid a high kinetic energy This energy is dissipated into heat, and since the passage time ($t_p$) through the slit is small (< 0 1 ms) the average energy density $\bar{\epsilon} = P/t_p$ is very high $\epsilon$ is the amount of energy dissipated per unit volume and per unit time its average in a homogenizer slit is typically $10^{11}$–$10^{12}$ W m$^{-3}$ Such high energy densities lead to very

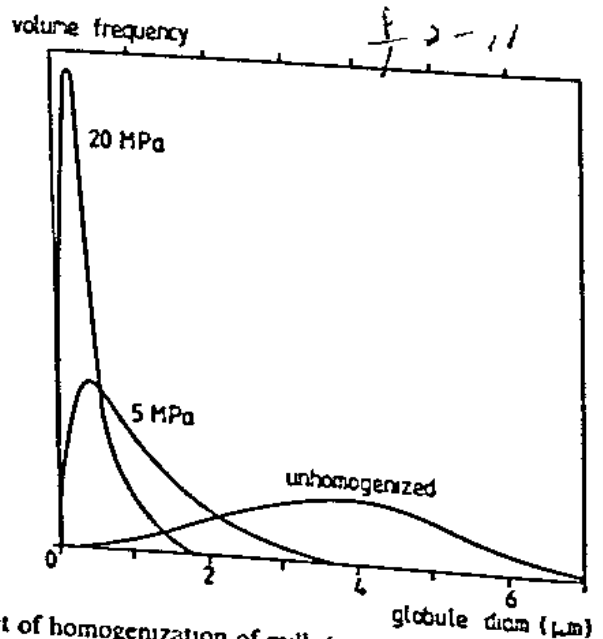



**Figure 14.8.** Effect of homogenization of milk (at a pressure of 5 or 20 MPa) on the volume frequency distribution of its fat globules After P Walstra *Neth Milk Dairy J* 29, (1975) 279

intense turbulence if the Reynolds number is sufficiently high; typically, the Reynolds number is some 40,000 in a production homogenizer. Turbulence implies that the flow has eddies, and these produce local fluctuations in liquid velocity and pressure. The pressure fluctuations can disrupt particles if the latter are fluid or not strong enough to withstand the stress. The energy density determines the size of and the velocity in the eddies, hence the local pressures and the distances over which they occur. Particles smaller than the smallest eddies generally cannot be disrupted. Table 14.2 gives examples of the effects created.

(Where the flow velocity in the valve slit is at its maximum, local pressure is less than 0. A negative pressure may cause cavitation (i.e., the formation and very sudden collapse of vapor bubbles). The collapse produces heavy shock waves, and these also may disrupt particles. The degree to which this happens in homogenizers varies. In most cases, globule disruption primarily will be caused by turbulent eddies.)

For a certain value of $\epsilon$ there will be a critical globule size $d_{cr}$; larger globules are disrupted, smaller ones are not. It follows from turbulence theory that $d_{cr}$ is proportional to $\epsilon^{-0.4}$. Conditions and consequently $\epsilon$ will vary throughout the slit in the homogenizer valve. This causes a wide distribution of globule sizes. It also implies that repeating homogenization under the same conditions decreases both average globule size ($d_{vs}$) and distribution width ($\sigma_s$). Besides disruption of globules, some coalescence will take place, and this also widens the size distribution.

**Table 14.2. Average Effect of Homogenization**

| Homogenizing Pressure (MPa)[a] | 0 | 5 | 10 | 20 | 40 |
|---|---|---|---|---|---|
| Temperature rise (K) | 0 | 1.2 | 2.5 | 5. | 10. |
| Diam. of smallest eddies ($\mu$m) | 0 | 0.31 | 0.24 | 0.18 | 0.14 |
| Number of fat globules ($\mu m^{-3}$) | 0.015 | 2.8 | 6.9 | 16. | 40. |
| Average diameter $d_{vs}$ ($\mu$m) | 3.3 | 0.71 | 0.47 | 0.31 | 0.21 |
| Maximum diameter[b] ($\mu$m) | 10.0 | 3.2 | 2.4 | 1.6 | 1.1 |
| Specific surface area ($m^2 \cdot ml^{-1}$ milk) | 0.08 | 0.38 | 0.56 | 0.85 | 1.25 |
| Distribution width $\sigma_s$ (−)[c] | 0.44 | 0.89 | 0.85 | 0.83 | 0.82 |
| Strokes parameter $H$ ($\mu m^2$)[d] | 20.00 | 2.20 | 0.87 | 0.36 | 0.16 |

*Note:* Approximate values for a milk with 4% fat.
Source: Mainly after H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, 1974.
[a] 1 MPa corresponds to 10 Bar, 9.87 atm, 10.2 kgf·cm$^{-2}$, and 145 psi.
[b] 99% of the fat is in smaller globules.
[c] Equation 12.15.
[d] Equation 12.14.

Several factors affect the result of homogenization Some of them are as follows

1. Type of homogenizer At the same pressure different types of homogenizer valves may cause quite different results; $t_p$ may differ and $\bar{\epsilon} = P/t_p$ Moreover, the spread in $\epsilon$ throughout the slit may differ, resulting in variation in $\sigma_s$ Figure 14 9 gives examples

   Other types of machines, such as high speed stirrers or ultrasonic generators, also may homogenize milk, but they are less effective They need a higher-energy input to obtain the same results as a high-pressure homogenizer Size distribution may be very wide

2. Homogenizing pressure This is the main variable, $\bar{\epsilon}$ is proportional to $P^{1\,5}$, because $\bar{\epsilon} = P/t_p$, and $t_p$ is proportional to $P^{-1\,2}$, as follows from equation 14 1 Under conditions of intense turbulence, $d_{cr}$ is proportional to $\epsilon^{-0\,4}$ and consequently to $P^{-0\,6}$ If distribution width is constant, the ratio of $d_{cr}$ to $d_{vs}$ is constant, and we obtain a similar relation for $d_{vs}$, hence

$$\log d_{vs} = \text{const} - 0\,6 \log P \qquad (14\,2)$$

   The constant varies among homogenizers, but the slope ($-0\,6$) does not (except for very small machines) (See Figure 14 9 ) The Stokes or sedimentation parameter $H$, important for creaming rate, being a diameter squared, follows

$$\log H = \text{const} - 1\,2 \log P \qquad (14\,3)$$

3. Two stage homogenization This should not be confused with homogenizing twice, it consists effectively of applying some back pressure at the exit of the first valve This tends to increase cavitation

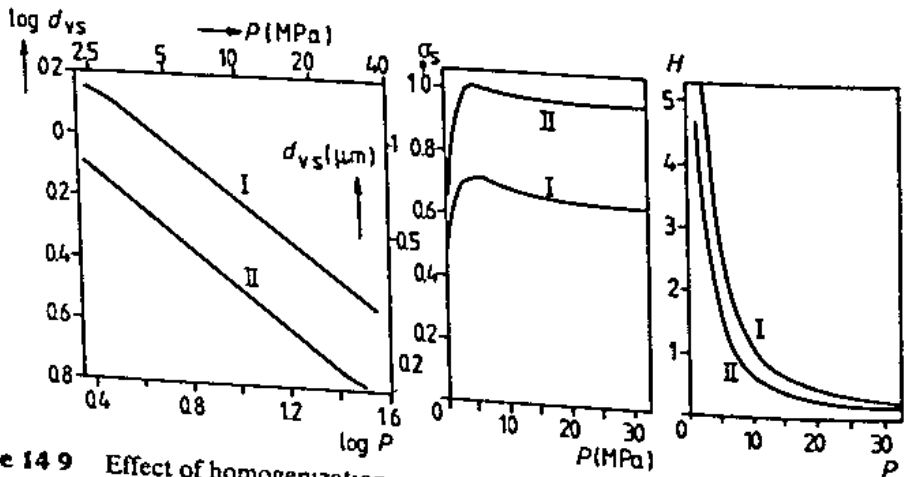



**Figure 14 9** Effect of homogenization pressure ($P$) on average globule size ($d_{vs}$) distribution width ($\sigma$) and Stokes parameter ($H$ in $\mu m^2$) for different homogenizers (I and II) After P Walstra *Neth Milk Dairy J* 29 (1975) 279

and may enhance slightly globule disruption. There is no significant homogenization in the second valve slit.

4. Fat content and ratio of surfactant to fat. These affect the extent of coalescence of the newly formed globules; they determine the probability that the latter collide before they have acquired a surface coat. In most milk products the surfactant (i.e., usually plasma protein) is relatively abundant. But in high-fat cream it is not so, and homogenization of such a product yields an average globule size and a distribution width higher than in homogenized milk.
5. Other product properties. The viscosities of fat and plasma and the nature of the surfactant present all affect the result somewhat.
6. Temperature. The effect of temperature relates particularly to fat crystallization. Globules with solid fat usually cannot be disrupted in a homogenizer. Figure 14.10 summarizes the effects of some variables.

### 14.4.2. New Surface Layers

Homogenization considerably enlarges the fat surface (Table 14.2) and thus creates denuded fat; the natural membrane material cannot spread out appreciably to cover a larger area, though most of it stays at the globules. The denuded fat is covered largely by plasma proteins, that means casein mi-

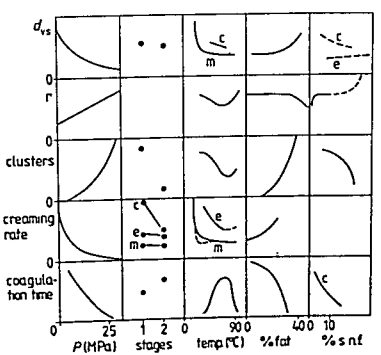



**figure 14.10.** The effect of conditions during homogenization on product properties (m, e, and e denote only for milk, cream, and evaporated milk, respectively). Average globule diameter ($d_{vs}$), thickness of surface layer (Γ, in mg·m$^{-2}$), degree of clustering, creaming rate, and time needed for heat coagulation are given as a function of pressure, one or two-stage homogenization, temperature, and contents of fat and solids-not-fat. Highly schematic; broken lines are less certain. From various sources.

celles, (partly) spread casein micelles, and whey proteins, as discussed in Section 12 1 2 Figure 14 11 depicts this new surface layer

The surface layer may not be formed by a mere adsorption of plasma proteins Spreading already has been mentioned Coalescence of newly formed globules can occur, causing a local decrease in surface area, which tends to compress the layer and to increase its thickness The transport of proteins to the fat surface is not caused by diffusion but largely by convection because of the very intense turbulence This causes an extremely quick coverage, calculated as less than 10 $\mu$s under most conditions It also causes a preferential adsorption of larger protein particles or molecules The rate of transport toward the fat is roughly proportional to protein concentration and to $(d_g + d_p)^3$, where g and p denote fat globule and protein particle, respectively This has several consequences

1. Casein micelles are adsorbed preferentially over whey proteins For example, casein may make up 93% of the dry mass of the surface layer and cover some 75% of the surface area
2. Large micelles are adsorbed preferentially over small ones
3. These differences are largest when $d_g$ is about equal to $d_p$ The smallest globules thus will have on average the heaviest surface layer

The layer is best characterized by the surface excess $\Gamma$ (i e , the mass of protein adsorbed per unit surface area) A rough average for $\Gamma$ in homogenized milk is 10 mg m$^{-2}$, but it differs among sites on one globule, among globules in one milk sample, and (largely because of difference in treatment) among samples It should be stressed that $\Gamma$ as given here pertains to the amount of dry protein per unit area Because the protein, particularly the casein, is present in a swollen state, the thickness of the layer (or its volume per unit area) is higher than the 7 nm corresponding to the $\Gamma$ of 10 mg m$^{-2}$ for a dry protein A rough estimate for the average thickness is 15 nm

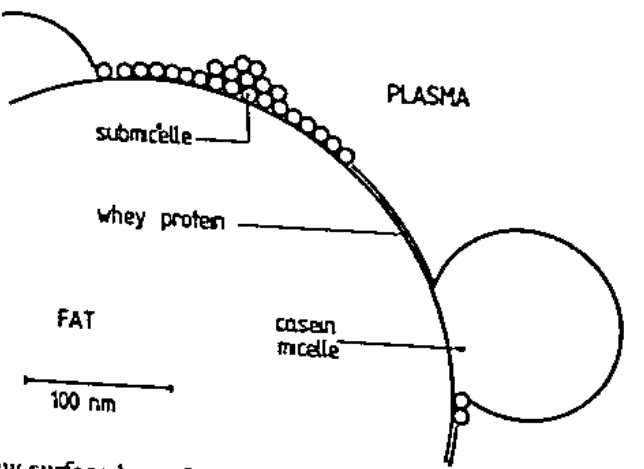



**Figure 14 11** New surface layer formed during homogenization Highly schematic example

The following factors affect Γ (see also Figure 14.10):

1. Factors affecting globule size, primarily homogenization pressure.
2. The rate at which a casein micelle can spread over the OW interface. This appears to increase with temperature; when homogenizing at 70°C, Γ is much lower than at 40°C.
3. Heating (e.g., 20 min at 80°C). This causes whey protein to associate with the casein micelles, and this whey protein no longer can cover the OW interface as such. Casein micelles may coalesce into large ones during heating; this happens in particular in evaporated milk. Consequently, Γ is higher when high-heated milk is homogenized, and evaporated milk may have globules with very thick layers.
4. The presence of surfactants that can replace proteins in the OW interface. This tends to lower Γ. (See Section 12.1.2.) Addition of sweet-cream buttermilk, monoglycerides, or Tweens before homogenization thus may lower Γ considerably.

The partial coverage of fat globules with casein makes them behave to some extent like large casein micelles. Any reaction that causes casein micelles to aggregate, such as renneting, acidification, or heating at very high temperatures, also will cause the homogenized fat globules to aggregate. Moreover, aggregation will be quicker, because the homogenization has increased effectively the content of micellar casein, the more so when fat content is higher. This has several consequences:

1. Sour products made of homogenized milk have different rheological properties from those of unhomogenized milk (Section 16.3); the same applies to cheese made from homogenized milk.
2. Homogenized cream may coagulate to some extent in coffee (i.e., show feathering). Feathering also depends closely on temperature, pH, and Ca content of the coffee.
3. Heat stability (i.e., the time or temperature of heating that a product can withstand before coagulating) is decreased; in cream, homogenization is the main variable influencing heat stability. (See Section 13.5.)
4. Before heat coagulation is noticeable, fat globules may aggregate to form clusters. These may cause excessive creaming.

All of these effects are about proportional to the amount of casein transferred from the plasma to the fat globules. (See further Figure 14.10.) The addition of surfactants before homogenization thus will increase stability to heat coagulation. On the other hand, it will decrease stability to coalescence. (See Section 14.2.2.)

### 14.4.3. Homogenization Clusters

During homogenization, when a partly denuded fat globule collides with another globule, one casein micelle may become attached to two globules at the same time, and a cluster is formed Such a cluster will be broken up again by turbulent eddies If, however, too little protein is available to cover the newly formed surface fully in the short time available, clusters will be formed again and finally may persist Then they probably are formed just outside the valve slit of the homogenizer, where turbulent intensity is too low to disrupt clusters but sufficient to let the partly denuded fat globules collide The new surface area $\Delta A$ is about proportional to $\phi P^{0.6}$, where $\phi$ is volume fraction of fat The specific area that can be covered by protein roughly equals [protein]/$\Gamma$ Consequently, fat and protein content, homogenization pressure, and the variables determining $\Gamma$ determine the degree of clustering (See Figure 14 10 ) The most important variable is fat-to-protein ratio A cream with less than 8% fat can hardly be made to cluster by homogenization, in a cream with more than 15% fat clustering usually cannot be avoided Clusters are disrupted again to some extent in a two-stage homogenizer, provided that the pressure drop in the second stage is much smaller than (e g , 30% of) that in the first stage, otherwise, new clusters would be formed The disruption of clusters leads to some coalescence

The clusters are often 10–100 $\mu$m in diameter, containing maybe $10^6$ globules Their formation can have several consequences

1. Viscosity of the products is increased, for instance, by a factor of 30 for 20%-fat cream (See Section 16 2 )
2. If the casein covered fat globules tend to aggregate, homogenization clusters do so at a higher rate Hence, some of the effects mentioned in the previous section are enhanced An 18%-fat sour cream, soured after homogenizing the cream at high pressure and low temperature, obtains a thick, pastelike consistency A 10%-fat homogenized cream, after souring, can be centrifuged to yield cream cheese and whey
3. When milk contains homogenization clusters it creams very quickly This can be achieved by separating the milk, homogenizing the cream, and mixing it again with the skim milk, such a product has been termed viscolized milk Homogenizing cream and mixing it with skim milk sometimes is applied to save on homogenization cost, it is called partial homogenization In order to prevent rapid creaming of clusters, the cream should contain at most 9% fat, and homogenization temperature should be high, say, 70°C

### 14.4.4. Other Effects

Homogenization of milk has a number of other effects

1. The color becomes whiter because of the increased scattering capacity (See Section 11 2 )

2. The tendency to foam increases. (See Section 15.2.)
3. The milk rapidly becomes rancid by lipolysis, unless sufficiently pasteurized. (See Section 5.5.)
4. The proneness to fat autoxidation, whether induced by light or by copper, is reduced. (See Section 5.6.)
5. Leukocytes, if not removed beforehand, are broken up and may give a visible sediment on standing.
6. The larger casein micelles are broken up if homogenization is done at high pressure. Compare their size, up to 0.3 $\mu$m, with that of the turbulent eddies. (See Table 14.2.) The fragments reassociate to much the original size within about 10 min. Otherwise, milk proteins hardly are affected by homogenization, apart from their adsorption onto and possible changes at the OW interface.
7. The cold agglutinin is inactivated. (See Section 14.3.)

### 14.4.5. Recombined Fat Globules

Milk fat can be emulsified into skim milk to make recombined milk. Usually, the (final) emulsification is done with a homogenizer. Consequently, the recombined fat globules are rather similar to homogenized ones, but they do differ in some respects. Recombined globules have a completely new surface layer, as depicted in Figure 14.11, while homogenized globules are partly covered with natural membrane material. Table 14.3 summarizes these and other differences among the globules in the various milks.

Of course, we can emulsify and homogenize milk fat in other liquids (e.g., buttermilk or whey), obtaining different surface layers that give the globules (hence the product) different properties. The fat also can be altered. (See further Section 17.7.)

## 14.5. CREAMING

Because of the difference in density between fat globules ($\rho_f \approx 920$ kg·m$^{-3}$ at room temperature) and milk plasma ($\rho_p \approx 1030$ kg·m$^{-3}$), the globules tend to rise. This causes creaming, which we often want to prevent, and it enables milk to be separated into cream and skim milk. Creaming is much enhanced when the globules have been aggregated into floccules, clusters, or granules. We will consider first the creaming of single globules.

From Archimedes's principle, the field force on a submersed spherical particle of diameter $d$ is $\frac{1}{6}a\pi d^3(\rho_p - \rho_f)$, where $a$ is the acceleration defining the field, whether from gravity or to centrifugation. The globule attains a velocity $v$ and, according to Stokes, the frictional force acting on the globule is $3\pi d\eta_p v$, where $\eta_p$ is the viscosity of the plasma (not of the milk). Putting both forces equal, we obtain the Stokes velocity

$$v_s = -a(\rho_p - \rho_f)d^2/18\eta_p \qquad (14.4)$$

**Table 14.3. A Comparison of the Properties of Natural (N), Homogenized (H), and Recombined (R) Fat Globules**

| Property | N | H | R |
|---|---|---|---|
| **Size Distribution** | | | |
| Average diam $d_{vs}$ ($\mu$m) | 3–5 | 0.2–1.0 | ≈ H |
| Stokes parameter $H$ ($\mu m^2$) | 15–40 | 0.2–4.0 | ≈ H |
| Surface area ($m^2$ $g^{-1}$ fat) | 1.3–2.2 | 7–34 | ≈ H |
| **Fat Composition** | | | |
| Difference between globules | yes | = N | no |
| **Fat Crystallization** | | | |
| Supercooling Needed (K) | ~ 20 | 25–35 | ≈ H |
| **Surface layers** | | | |
| Main component | lipoprotein | both | caseinate |
| Natural membrane (% of area) | almost 100 | 5–40 | 0 |
| **Fat Deterioration** | | | |
| Lipolysis | little | >> N | ≈ H |
| Autoxidation | prone | < N | < H |
| **Changes in Dispersion** | | | |
| Globule disruption | possible | << N | ≈ H |
| Cold agglutination | yes | < N | ≈ H |
| Heat coagulation | no | possible | > H |
| Coalescence | possible | << N | < H |

For gravity creaming $a$ equals $g$ (9.81 m $s^{-2}$), in a centrifugal field $a = R\omega^2$, where $R$ is effective radius of the centrifuge and $\omega$ is angular velocity in radians per second. In a milk separator $a$ is usually about 4000 × $g$.

Numerous conditions must be fulfilled for equation 14.4 to hold. We will mention only a few.

1. The concentration of fat globules must be very low, say, $\phi < 10^{-3}$. This is rarely the case.
2. Creaming may not be disturbed by Brownian motion or convection currents. Disturbance always occurs for small (say, < 1 $\mu$m) fat globules during gravity creaming.
3. Globules should be smooth spheres. This is not true for most homogenized fat globules. (See Figure 14.11.)

Nevertheless, Stokes's equation is useful as a reference and to show the approximate effect of several variables.

Because the fat globules in a sample differ in size, the proportion $q$ of the fat reaching the cream layer per unit time may be calculated from

$$q = \Sigma N_i d_i^3 \, v_i / D \, \Sigma N_i d_i^3 \tag{14.5}$$

where $D$ is the depth of the layer of milk. Assuming equation 14.4 to hold, we have $v = v_s$, thus proportional to $d^2$. This makes $q$ proportional to $H = S_5/S_3$, equation 12.14. Combining equations 12.14 and 14.5 with 14.4, we obtain for the case of gravity creaming

$$q \approx 470 \, (\rho_p - \rho_f) H / \eta_p D \tag{14.6}$$

as the fraction of the fat creaming per day, when the other variables are in S.I. units. The equation only holds as long as $H$ is virtually unaltered; as soon as the milk just below the cream layer becomes depleted of the larger globules, $q$ decreases. Usually, $q$ remains roughly constant as long as total creaming ($Q = \int_0^t q \, dt$) is below 0.4.

In practice, the following factors mainly determine the extent of creaming:

1. Local acceleration, duration of creaming, and distance over which creaming must occur. In a separator these factors may vary considerably among globules in different sites.
2. Globule size, not only because of equation 14.4, but also because small globules are disturbed most by Brownian motion and convection currents. In homogenized products, the smallest globules have the thickest surface layers; their density may even be higher than that of the plasma, causing them to sediment downward.
3. Fat content. (See Figure 14.12.) For gravity creaming, the mutual hindrance of globules disturbs their rising very much; for certain conditions, the average rising velocity was found to be $(1 - \phi)^{8.6}$ times the Stokes velocity. For 7% fat (w/w) this reduces creaming rate to half of the Stokes rate. In centrifugal creaming the relation is different, and up to 7% fat the deviation from equation 14.4 is small; the explanation is outside the scope of this book.
4. Temperature affects primarily $(\rho_p - \rho_f)/\eta_p$. (See Figure 14.12B and the appendix, Table A.22.) $\rho_f$ increases when part of the fat crystallizes. Consequently, supercooled fat globules cream somewhat faster than partially crystallized globules at the same temperature.
5. Addition of thickening agents, usually polysaccharides like locust bean gum or carboxymethyl cellulose. These increase $\eta_p$; hence they slow down creaming. Heating of evaporated milk also increases $\eta_p$. It should be realized that the relevant viscosity is that at the low velocity gradient around rising globules, and that may be higher than the viscosity as measured in a viscometer where the velocity gradient

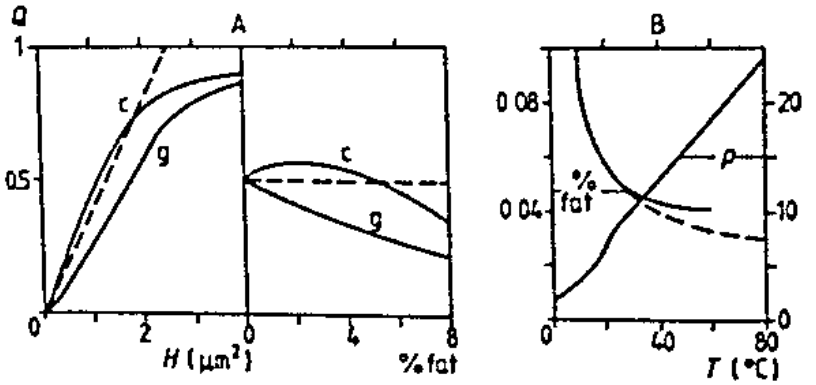



**Figure 14 12.** Creaming of single fat globules (A) High pasteurized milk creaming under gravity (g) or in a centrifuge (c) as a function of Stokes parameter ($H$) and fat content, conditions were taken so that the products of acceleration and creaming duration were equal, $Q$ = fraction of the fat creamed (B) Efficiency of separation in a milk separator; fat content of skim milk and $p = (\rho_p - \rho_f)/\eta_p$ as a function of separation temperature Approximate examples The broken lines are predictions according to equation 14 6 Mainly after P Walstra and H Oortwijn, *Neth Milk Dairy J* 29 (1975) 263

is higher In some cases, particularly when adding κ-carageenan, the polysaccharide even may form (either alone or in combination with the casein micelles) a weak network that entraps the fat globules Cream rising then is virtually absent

If we want as little creaming as possible, the larger globules are the ones that interfere For a certain average globule size ($d_{vs}$), the sedimentation parameter ($H$) will be larger if the distribution is wider ($\sigma_s$ larger) To predict creaming during standing of the product, we may use a centrifugal test, the difference between curves g and c in Figure 14 12A gives some indication of the value of such a test Approximate examples of creaming rate in some products are in Table 14 4

In the centrifugal separation of milk, we usually want as little fat left in the skim milk as possible, and the smaller globules interfere Because creaming takes so little time (about 3 s), and because the milk is streaming vigorously on entering the separator, cold agglutination of fat globules can be neglected Usually, Stokes's law applies fairly well The greater the fraction of fat in small globules and the wider the spread in conditions (see point 1, above), the higher the fat content of the skim milk Figure 14 12B gives an example as a function of temperature, the difference between the values found and predicted (broken line) probably results from disruption of fat globules in the separator at high temperature Any disruption of fat globules during handling will increase the fat content of the skim milk

Creaming of single globules may occur in high pasteurized milk, in raw or low pasteurized milk if the temperature is above around 30°C, and in most homogenized milks In these cases the cream contains few small globules and the skim milk almost no large ones The cream layer is relatively thin, it usually contains 40–50% fat This fat content is still much below that for

**Table 14.4. Creaming Rate in Some Milk Products**

| Product | $(\rho-\rho_f)/\eta_p$ (ks·m$^{-2}$) | $H$ ($\mu$m$^2$) | $D$ (cm) | $q$ (% per Day) Eq. 14.6 | $q$ (% per Day) Corrected[a] |
|---|---|---|---|---|---|
| Pasteurized milk | 50. | 10.–50. | 20 | 12.–60. | 8.–40. |
| Homogenized milk | 50. | 0.8–1.5 | 20 | 0.8–1.7 | 0.5–1.1 |
| UHT milk | 40. | ~ 0.2 | 10 | ~ 0.4 | ~ 0.2 |
| Evaporated milk | 2.5 | ~ 0.4 | 5 | ~ 0.1 | ~ 0.03 |
| Condensed milk[b] | 0.07 | 4.0[c]–50. | 5 | 0.03–0.30 | 0.01–0.12 |

*Note:* Approximate examples of the percentage of the fat reaching the cream layer, calculated for creaming of single globules at constant temperature (~ 20°C) in containers of height $D$.

[a]Rough estimate, taking into account effects of fat content and of thick surface layers.
[b]Sweetened.
[c]Slightly homogenized.

the closest packing of undeformed spheres (about 70%; compare Figure 14.7). Nevertheless, many globules almost touch each other, and this may give rise to partial coalescence, particularly when part of the fat crystallizes. Consequently, the cream often is not redispersed easily.

Any clustering, whether caused by homogenization or by heat coagulation of homogenized fat globules, will enhance creaming considerably. The size of a cluster compared to the size of a single globule has a much larger effect on rising rate than the decreased density difference; a cluster of one hundred globules would probably rise fifteen to twenty times as fast as a single globule.

The creaming of raw or low-pasteurized milk in the cold is determined effectively by the flocculation of the globules by agglutinin. (See Section 14.3.) The main variables affecting this "natural" creaming are summarized in Figure 14.13. There are large differences among lots of milk. Natural

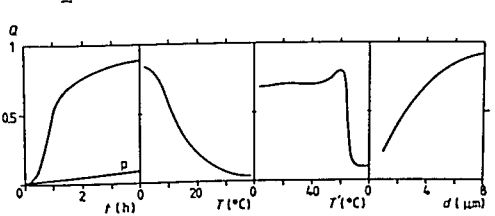



**Figure 14.13.** Gravity creaming in cold raw milk. Effect of creaming time ($t$), creaming temperature ($T$), temperature of prewarming ($T'$ during 30 min) and fat globule size ($d$) on the fraction creamed (Q). p = high-pasteurized milk. Approximate examples from various sources.

creaming leads to a deep cream layer, containing relatively little fat, generally about 20%. The cream can be dispersed easily throughout the milk by shaking or stirring. The fat content of the skim milk is usually between 0.1 and 1.0% if the creaming temperature is low (e.g., 5°C). Because the rising flocculs comprise large and small globules, the difference in average globule size between cream and skim milk is relatively small. (See Figure 14.13.)

## *Suggested Literature*

Fundamental aspects of emulsions, including homogenization, are discussed in

P. Becher, ed., *Encyclopedia of Emulsion Technology*, vol. 1 (New York: Dekker, 1983).

Several aspects of this chapter are treated in more detail by

H. Mulder and P. Walstra, *The Milk Fat Globule. Emulsion Science as Applied to Milk Products and Comparable Foods* (Wageningen: Pudoc, 1974).

Particulars on the size distribution of milk fat globules are given by

P. Walstra, *Neth. Milk Dairy J.* 23 (1969): 99–123.

Homogenization's effect on fat globule size was studied by

J. D. S. Goulden and L. W. Phipps, *J. Dairy Res.* 31 (1964): 195.

P. Walstra, *Neth. Milk Dairy J.* 29 (1975): 279–94.

Surface layers formed by homogenization or recombination were studied by

H. Oortwijn and P. Walstra, *Neth. Milk Dairy J.* 33 (1979): 134–35.

The creaming of milk fat globules is discussed by

P. Walstra and H. Oortwijn, *Neth. Milk Dairy J.* 29 (1975): 263–78.

# 15

# Interactions of Milk With Air Bubbles

Milk and milk products interact frequently with air bubbles. Milk products may foam; sometimes it is a nuisance, as in the handling of separated milk; sometimes it is desired, as in the whipping of cream. Classical churning is based largely on interaction of cream with air bubbles. The milk proteins and the fat globules mainly are involved in the interaction, and they are changed by the contact with air. This is discussed in Section 12.1, which should be read for a better understanding of the present chapter.

## 15.1. GENERAL ASPECTS OF FOAMING

A liquid can be made to foam by beating in air, but only if the liquid contains a surfactant. Otherwise, the layer of liquid between two air bubbles would flow away immediately, almost falling down as a drop would do. But if a surfactant is present it adsorbs onto the air–liquid interfaces, and now the liquid flow produces an interfacial tension gradient. This gradient exerts a stress on the liquid, and the interfaces now act to some extent as solid walls, considerably retarding the downflow of the liquid between the bubbles. In this way, the bubbles formed persist long enough to form a foam.

A foam also can be made by generating bubbles in the liquid, for instance, by injection, or by expansion if a gas has been dissolved in the liquid under pressure. In these cases, the above paragraph does not apply precisely, but a surfactant still is needed to avoid immediate collapse of bubbles reaching the liquid surface. The sequence of events is usually as shown in Figure 15.1, regardless of the method of making the foam. But the extent of foaming

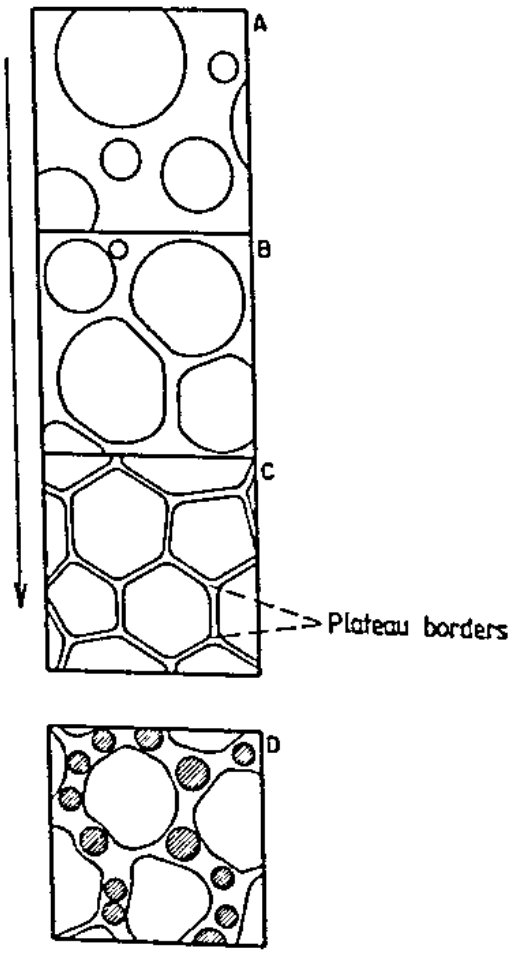



**Figure 15.1.** Formation and structure of a foam Schematic (A) Bubbles of various size are formed by beating air into the liquid (a bubble foam) (B) Bubbles rise start to deform each other liquid drains away a polyhedral foam forms Small bubbles dissolve (C) Drainage continues and bubbles coalesce until the foam is fairly homodisperse (D) With solid particles the final air content of the foam is much lower From H Mulder and P Walstra *The Milk Fat Globule* (Wageningen Pudoc 1974)

and the stability of the foam may depend profoundly on the method of making the foam, for instance, very fast beating itself may destroy the foam to some degree

A foam made from an aqueous liquid has much in common with an oil-in-water emulsion, both are dispersions of an apolar fluid phase in a polar liquid There are, however, several differences in practice The interfacial tension between the two phases is relatively high, say, 40 mN m $^{-1}$, roughly ten times that in emulsions Consequently, the Laplace pressure tends to be higher (Section 12 1 3), and small bubbles are thus difficult to make Moreover, they rapidly dissolve again (Ostwald ripening) because of the overpressure and the solubility of air in water As a result, the bubbles are large, and since they differ much in density from the continuous phase they rise rapidly, according to Equation 14 4 mostly at a rate of at least 1 mm s$^{-1}$ The bubbles rapidly form a foam layer on top, and the upward force that still acts on them causes mutual deformation Liquid flows away between bubbles, and they assume a polyhedral shape Between two bubbles there is now a flat film or lamella, bordered by channels (Plateau borders) where three lamellae meet

A foam may change in various ways

1. Liquid may drain from the foam by gravity. Drainage is, of course, hindered by viscous resistance, the more so as the lamellae become thinner, and the liquid drains ever slower. Ultimately, the foam will contain very little liquid; when lamella thickness is about 1% of bubble diameter, the volume fraction $\phi$ of air in the foam may be as high as 0.95. In practice, we often express air content as percentage overrun equal to $100\ \phi/(1-\phi)$. Drainage can be retarded by increasing the viscosity of the liquid, but when a not too low $\phi$ is desired a higher viscosity postpones the problem rather than solving it. We can try to give the liquid a yield stress; the continuous phase is liquid during beating, but "solidifies" after the foam is made. Another method is to provide the liquid with solid particles that act as spacers. (See Figure 15.1). A prerequisite is that the contact angle (as defined in Figure 12.2) be less than 90°; otherwise, the particles would cause the film between the bubbles to break. The casein micelles of milk may act as spacers, and this may be one reason why the foam of skim milk usually has $\phi \leq 0.85$ ($\sim$ 600% overrun). Fat globules can act as spacers and in whipped cream mostly $\phi$ is about 0.5.
2. Evaporation of water from the foam causes the lamellae to thin, often quicker than by drainage. Evaporation generally occurs at the top of the foam where the lamellae are already thinnest because of drainage.
3. Rupture of the lamella may occur. This is not the place to discuss the stability of thin films; properties and concentration of the surfactants present are paramount. Broadly speaking and other things being equal, a thinner film ruptures more easily. A foam contains relatively few bubbles (as compared to the number of droplets in an emulsion), so not many films have to rupture to cause a profound change. When a lamella (i.e., a film within the foam) breaks, two bubbles coalesce, and the foam becomes coarser without losing volume. When films between the upper bubbles and the free air break, the foam collapses; this is what happens generally, because the films at the top of a foam are thinnest. Fat globules also can cause lamellae to break, as is discussed in the next section.
4. Ostwald ripening goes on, which means that small bubbles disappear and the remaining ones grow larger. The foam becomes coarser, containing fewer lamellae, and thus becomes less stable. Ostwald ripening can be stopped, or at least much retarded, by the following mechanism. When air dissolves from a bubble, its surface area decreases. Hence, the surface excess $\Gamma$ tends to increase and $\gamma$ to decrease. This causes, in turn, a lower Laplace pressure, hence a decreased tendency for air to dissolve. If the relative decrease in $\gamma$ equals the relative decrease in bubble radius, dissolving of air stops. (The condition is that $-\mathrm{d}\gamma/\mathrm{d}\ln A = \gamma/2$, where $A$ is area.) A prerequisite, however, is that the surfactant is not desorbed from the AW interface. Now, if

there is Gibbs equilibrium (equation 12 1), the surfactant does desorb, hence, no increase in $\Gamma$ and no decrease in $\gamma$ on bubble shrinkage Several proteins, however, do not desorb or do so slowly, and now the stabilizing mechanism acts Protein solutions, therefore, may give fairly stable foams

It may be concluded that it is very difficult to make a stable foam Its lifetime is often a matter of minutes or hours, rather than months as it is for many emulsions In practice, many foams are made stable by making them rigid, for instance, by solidification of the continuous phase, or by forming a network of solid particles around the bubbles, this prevents Ostwald ripening, lamella rupture, and mostly drainage

## 15.2. FOAMING OF MILK PRODUCTS

Preparations of milk proteins may stabilize foam Caseinates may do so because they are strongly adsorbed onto AW interfaces, presumably because of the easy accessibility of hydrophobic portions of the molecules. The foaming properties of whey proteins depend greatly on conditions. (See further Section 17 8 2 )

Milk, however, contains a mixture of proteins Moreover, casein is present in micelles, and they adsorb as such onto the air bubbles When the bubbles shrink because of dissolution of air, the micelles stay in the interface, and other adsorbed substances presumably desorb Further shrinkage causes the micelles to touch each other and form a coherent layer, and finally the air dissolves completely and an empty, folded sac consisting of a membrane of casein micelles remains Such "ghost membranes are found particularly in skim milk that has been foaming at temperatures above 20°C At low temperatures the ghost membranes do not form and it is striking that foam stability also is low (See Figure 15 2)

Fat globules make a foam much less stable Presumably, they occasionally pierce or make contact with the AW interface when caught in a foam lamella Now, membrane material and possibly liquid fat spread over it (Section 12 1 4), and the spreading causes rupture of the film Fully liquid globules will not pierce the AW interface easily The globule and the adsorption layer on the AW interface repel each other When the lamella becomes thinner than the globule diameter, the globule tends to be deformed It will be recalled that $\gamma$ of the globules ($< 2$ mN m$^{-1}$) is much lower than $\gamma$ of the bubbles ($\sim 50$ mN m$^{-1}$) so that the globules can be flattened by the Laplace pressure exerted by a bulging lamella But globules with solid fat cannot be flattened, and they may pierce the interface the more readily the larger they are Homogenization diminishes the tendency of the globules to make a foam unstable, possibly also because of the different surface layer These aspects are illustrated in Figure 15 2 The large effect of fat content is striking, the

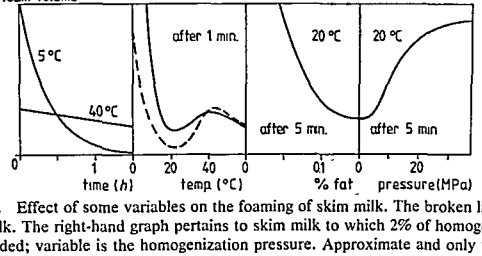



**Figure 15.2.** Effect of some variables on the foaming of skim milk. The broken line pertains to whole milk. The right-hand graph pertains to skim milk to which 2% of homogenized milk has been added; variable is the homogenization pressure. Approximate and only to illustrate trends. Mainly after H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

more efficient the centrifugal separation, the larger the tendency of skim milk to foam. The effect of temperature is complicated; fat crystallization, casein micelle properties, and possibly other factors play a part.

Polar lipids, like phospholipids, monoglycerides, and fatty acids, depress foaming. Consequently, addition of buttermilk and lipolysis diminish the foaming tendency of milk.

Heating to such an extent that whey proteins become heat denatured causes milk to yield a more voluminous and more stable foam on beating. Heating at sterilization temperatures diminishes foaming capacity. Slightly lowering the pH increases tendency to foam, but a further lowering decreases it very much. Evaporation of skim milk increases its foaming capacity; a concentrate of 30% dry matter that has been vigorously homogenized can give a stable foam with $\phi$ of about 0.8 (400% overrun).

Finally, it should be stressed that the precise extent of the above-mentioned effects may depend on the way the foams are made.

## 15.3. CHURNING AND WHIPPING

While the preceding section dealt with air bubbles in low-fat products, the main theme of this section is the interaction of cream with air bubbles. A newly formed bubble has a clean surface for a very short time, and before a protein adsorption layer has formed some fat globules become attached to the surface and are held there by surface forces. (*See Section 12.1.4.*) When the air bubbles form a foam layer on top of the cream, the foam is enriched in fat. By analogy to the collection of ore particles in a foam, this process may be called flotation.

In practical situations, beating often goes on in such a way that a foam layer is not formed. Subsequent events depend on conditions, the crucial one being the solid–liquid ratio of the fat. It will be recalled that this ratio

depends on fat composition, temperature, and possibly temperature history and globule size (See Section 5 7) If there is very little liquid fat, globules only become attached to the bubbles and usually stay there If the fat is fully or predominantly liquid, a thin layer of liquid fat may spread from the attached globule over the air bubble, this layer is disrupted when the bubble is broken up during subsequent beating Minute fat droplets result, and this often is called colloidal fat, it is so finely dispersed that it escapes centrifugation (at, e g , 5000 × *g*) If distribution of solid and liquid fat is more even, part of the latter spreads over the air bubble, and a somewhat smaller globule remains When the surface area of the bubble diminishes, for instance, because it coalesces with another bubble, the attached fat globules are driven nearer to each other When this happens, the globules associate into granules, held together by the liquid fat originally spread over the surface, because the liquid fat is not covered by adsorbed protein at the air side of the bubble, it can readily act as a cementing agent

Another important variable is fat content If it is low, the fat globules primarily will make the foam unstable as discussed in the previous section, for a suitable solid–liquid ratio granules may form If fat content and the solid–liquid ratio are high, the fat globules may cover the surface of the air bubbles almost fully, thus making them stable

Figure 15 3 illustrates the various processes occurring during *flotation churning* (i e , a churning process in which incorporation of air is essential) The tendency of the cream to foam goes through a maximum The increasing quantity of colloidal fat, which includes a large proportion of polar lipids derived from the fat globule membrane, makes the foam unstable During the latter stages of the churning, the small granules formed by flotation collide and form larger granules because of the agitation, this may be called mechanical clumping, as no air bubbles are needed Apparently, it needs a somewhat higher proportion of liquid fat than proper flotation churning

Generally, churning is much faster when the liquid fat content is higher But we usually are interested not only in speed but also in completeness of churning In other words the fat content of the buttermilk should be low, the more so as a large part of the fat in buttermilk cannot be separated easily If there is much liquid fat, a large fraction of it is lost in the buttermilk If the fat is fully liquid treating cream in a churn does not lead to formation of butter granules but to homogenization

The effect of some variables is shown in Figure 15 4 Other factors play a part, for instance, pH and precooling A complete picture is difficult to obtain, because each variable influences the effects of the others

The *whipping of cream* is very similar to churning and if the liquid fat content is too high churning does occur (i e , visible granules of fat form and the foam breaks) This is often a problem in whipped cream Figure 15 5 illustrates events during proper whipping The amount of foam (overrun) increases only slowly The fat globules have a strong destabilizing effect on the foam as was discussed before The air bubbles, to be stable, should be

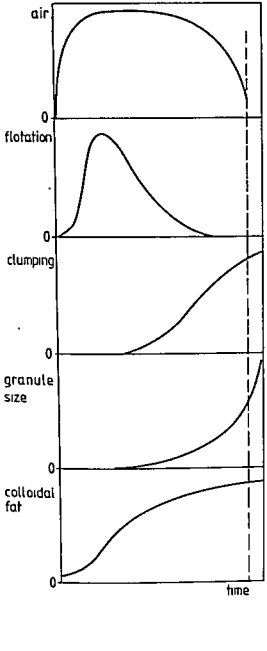



**Figure 15.3.** Traditional churning of cream. Amount of air in the cream, rate of flotation (rate at which fat globules and small granules attach to air bubbles), rate of mechanical clumping; size of granules, amount of fat in the colloidal state (i.e., not recoverable by centrifugation) during the course of churning. The broken line indicates the point of breaking. The figure is meant as an illustration only and is not exact. From H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

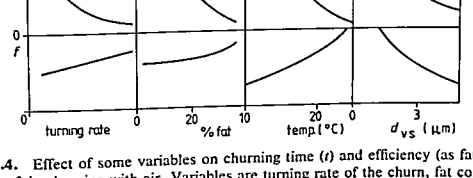



**Figure 15.4.** Effect of some variables on churning time ($t$) and efficiency (as fat content of buttermilk, $f$) in churning with air. Variables are turning rate of the churn, fat content of the cream, churning temperature, and average fat globule size ($d_{vs}$). Only meant to illustrate trends. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

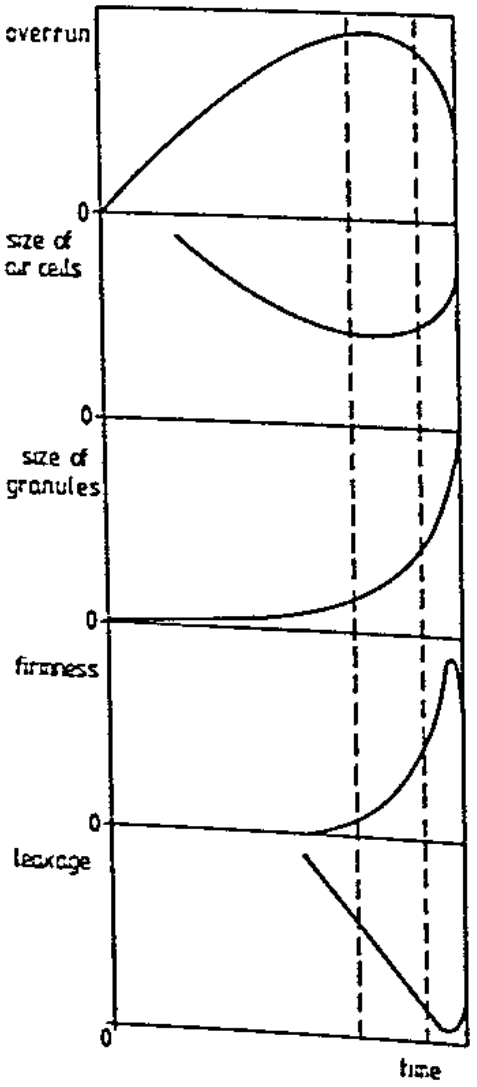



**Figure 15.5.** Processes taking place during whipping of cream The parameter of firmness could be the time needed to lower a weight into the product leakage means the amount of liquid drained from a certain volume in a certain time Between the broken lines the product is acceptable Curves are only approximate After H Mulder and P Walstra, *The Milk Fat Globule* (Wageningen Pudoc 1974)

covered almost fully by attached fat globules A fairly high fat content is needed to achieve this, though not as high as is common in whipping cream (about 35%) If fat content is lower, however, plasma will drain from the foam, a process usually called leakage To obtain a really stable foam that can also withstand some manipulation (e g , shaping or stirring), the fat globules should aggregate into granules to some extent, making a three-dimensional structure throughout the liquid in the foam and thereby mechanically stabilizing the air bubbles The whipping process thus should strike a balance between foaming and churning Too little churning gives a less stable foam, too much gives a coarse and granular foam or even segregation into granules and buttermilk An important variable is the intensity of beating Vigorous beating rapidly gives a foam with small air cells and a high overrun Slow beating easily leads to churning

It is also possible to make a foam in which the fat globules are not aggregated They act predominantly as spacers in the foam lamellae (Figure 15 1) and may or may not be attached to the bubbles It is often necessary

to homogenize the cream and add surfactants. The whipped or foamed product differs from ordinary whipped cream. It has a high overrun, is not very stable to handling (it partially collapses on agitation), and shows considerable Ostwald ripening.

## 15.4. ICE CREAM

Ice cream mix typically contains 10.0% milk fat, 11.0% milk solids-not-fat, 14.0% added sugar, and 0.3% additives like emulsifier, stabilizer, and flavor and color substances. The mix always is homogenized. It is made into ice cream by "freezing," which means that it is rapidly cooled to $-4°$ to $-6°C$ while beating in air. These processes must take place simultaneously; after freezing, air cannot be beaten in, and beating without freezing does not give a stable foam. General aspects of freezing milk are discussed in Section 17.4.

The beating and cooling process is to some extent similar to the whipping of cream. Some liquid fat spreads over the air bubbles, and granules of fat globules form. But granule formation will be largely mechanical clumping, because it goes more rapidly at a lower temperature, and a lower temperature means less liquid fat. But it also means more ice crystals, and these damage the fat globules during beating.

An intricate structure is formed, and the structural elements are ice crystals, air cells, fat globules, fat granules, and often lactose crystals; many fat globules and granules are attached to the air cells. The sizes are very approximately as follows:

ice crystals $\sim$ 50 $\mu$m (or smaller if freezing is very rapid)
lactose crystals $\sim$ 20 $\mu$m
air cells 60–150 $\mu$m
foam lamella thickness 10–15 $\mu$m
fat globules $< 2$ $\mu$m
fat granules 5–10 $\mu$m

The size of the fat globules and the degree to which granules have formed largely determine the size of the air cells. If the mix is not well homogenized, or if large homogenization clusters are present, the ice cream obtains a rather coarse texture with large air cells. Homogenization, however, diminishes the susceptibility of the globules to coalesce (Section 14.2), and some partial coalescence (granule formation) is needed. The structures of partially coalesced globules and their attachment to the air cells give the ice cream a residual firmness after the ice crystals have melted. This is important during eating. The texture imparted by the structures mentioned, often designated as dry, also is needed to prevent excessive sticking of the ice cream to processing equipment. To enhance coalescence, an emulsifier is added to

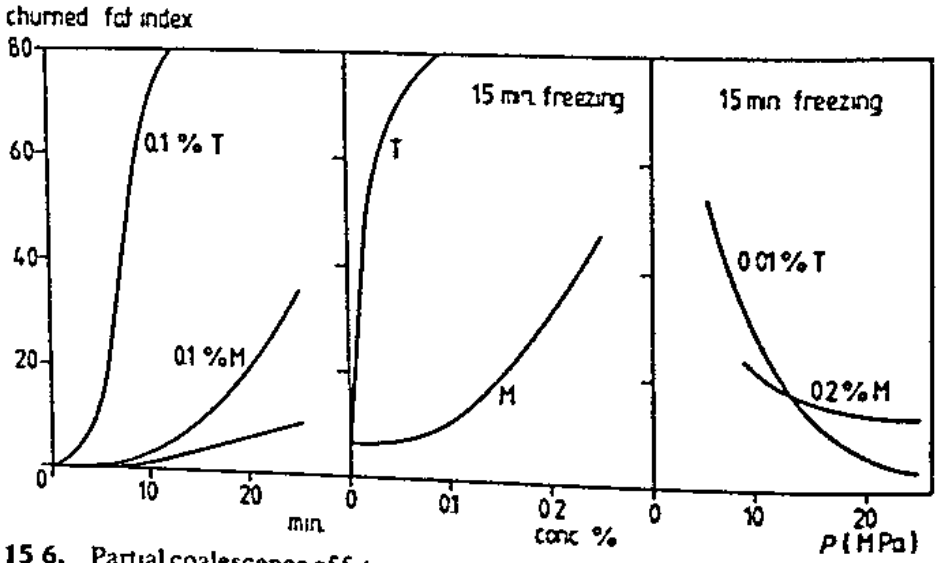



**Figure 15 6.** Partial coalescence of fat in ice cream Churned fat index expressed as percentage decrease in turbidity, as a function of freezing time (min), presence and concentration of glycerol monostearate (M) or Tween 80 (T), and homogenizing pressure (*P*, MPa) Results are only approximate as they depend on several factors After H Mulder and P Walstra, *The Milk Fat Globule* (Wageningen Pudoc, 1974)

the mix before homogenization The effect of some variables on partial coalescence, as indicated by a churned fat index, is shown in Figure 15 6 Too much partial coalescence leads to a coarse and grainy texture

The components of the ice cream have the following main functions

1. Fat is needed for the taste but also to form the structures of partially coalesced globules just discussed
2. Milk solids-not fat are needed for the taste, to lower the freezing point, and to increase the viscosity of the remaining liquid, which impedes drainage of liquid The protein is needed to form surface layers around the fat globules on homogenization and around air bubbles on beating
3. Sugar lowers freezing point, increases viscosity, and gives a sweet taste
4. The stabilizer is usually a polysaccharide like alginate, carrageenan, locust bean gum, or carboxymethyl cellulose, sometimes gelatin is used It increases the viscosity or even gives the melted ice cream gel-like properties Too high a stabilizer content, however, gives an undesirable mouthfeel
5. Emulsifier is not needed to emulsify the fat or to stabilize the foam It makes the fat globules less stable (Section 14 2 2) so that they can form granules during beating
6. Ice crystals are essential for consistency and coolness, the low temperature lessens the sweetness The ice crystals should not be too large, to avoid a sandy mouthfeel

7. The air cells have three main functions. They make the ice cream light; it would be too rich without air. They make the ice cream soft and thereby deformable during eating. They isolate against excessive coolness; the ice cream would be too cold in the mouth without air. Mostly, air content is about 50% by volume.

## *Suggested Literature*

General aspects of foaming are in a symposium report:

R. J. Akers, ed., *Foams* (New York: Academic Press, 1976).

Most of the material in this chapter is discussed in more detail by

H. Mulder and P. Walstra, *The Milk Fat Globule: Emulsion Science as Applied to Milk Products and Comparable Foods*. (Wageningen: Pudoc, 1974).

Physical aspects of ice cream are discussed by

K. Berger, in *Food Emulsions*, S. Friberg, ed. (New York: Dekker, 1976), pp. 142–213.

# 16

# Rheological Properties

Many liquid milk products behave under many conditions as Newtonian liquids, this implies that they have a true viscosity that does not depend on the shear rate Viscosity ($\eta$) is defined as the ratio of shearing stress ($\tau$) to shear rate, this pertains to simple shear flow (i e , laminar flow with parallel streamlines) Shear rate is then equal to the velocity gradient $G$, which is perpendicular to the direction of flow In other types of flow other relations hold, but we will not consider these

We will discuss first Newtonian behavior, and in the next section the exceptions for liquid milk products, which then leads into milk gels Finally, milk fat, which is more like a solid, is discussed briefly Some aspects of its rheological behavior are paralleled to some extent in other products

## 16.1. NEWTONIAN BEHAVIOR

For dispersions in which the particles do not attract or repel each other, the increase in viscosity caused by the particles results only from hydrodynamic interaction Viscosity usually is well described by the semiempirical Eilers equation

$$\eta = \eta_o \left(1 + \left(\frac{1\,25\,\phi}{1 - \phi/\phi_{max}}\right)\right)^2 \qquad (16\,1)$$

which reduces to the well known Einstein equation ($\eta/\eta_o = 1 + 2\,5\,\phi$) for $\phi \to 0$ Here $\phi$ is the volume fraction of the dispersed particles, and all particles that are at least an order of magnitude larger than the molecules of the liquid (i e , water) must be considered This means that even lactose molecules should be included $\phi$ pertains to the hydrodynamic volume, and if the particles are nonspherical, it is larger than the geometrical volume,

moreover, the hydrodynamic volume includes hydration water, "hairy" layers, and cavities in particles. We thus have for milk

$$\phi \approx \phi_f + \phi_c + \phi_w + \phi_l \tag{16.1a}$$

$\phi_f$ relates to fat globules, whose volume is 1.11 ml·g$^{-1}$ lipid in fat globules at room temperature; $\phi_c$ relates to casein micelles (~ 3.9 ml·g$^{-1}$ of dry casein); $\phi_w$ relates to whey proteins (probably on average ~ 1.5 ml·g$^{-1}$ of dry protein); $\phi_l$ relates to lactose (~ 1 ml·g$^{-1}$ of lactose).

$\phi_{max}$ is the hypothetical volume fraction that would give close packing of all of the particles, assuming them to be present in the same proportion (i.e., with the same size distribution). Because the particles are so different in size, $\phi_{max}$ will be high. $\phi_{max}$ equal to 0.9 seems a reasonable estimate for milk products, but for evaporated milk it is perhaps a bit higher and for high-fat cream a bit lower. Although the size of the particles as such does not come into the equation, the spread in size thus may affect it.

$\eta_o$ is the viscosity of the liquid in which the particles are suspended, hence water with some other small molecules. For milk, $\eta_o$ is about 1.02 $\eta$(water); for evaporated milk it may be slightly higher.

Equation 16.1 fairly well predicts the viscosity of skim milk, milk, cream, concentrated milks, and milk ultrafiltrate up to $\phi = 0.6$. (See, however, Section 16.2.) Examples of viscosities are in Figure 16.1. Total $\phi$ should be used, not just one of its fractions. Taking cream as an example, using $\phi_f$ as the variable and $\eta_o$ as the viscosity of milk plasma would give too low results (e.g., a 20% too low $\eta$ for 35%-fat cream), because $\phi - \phi_f \approx 0.16$. Beyond $\phi = 0.6$, corresponding with $\eta/\eta_o \approx 10$, the viscosity increases steeply and flow usually starts to deviate significantly from Newtonian behavior. The deviations may result from interaction forces between the particles and from their deformability. Ultrafiltered or concentrated whey seems to give significant deviations from equation 16.1 at a much lower $\phi$, even if the fact that the hydrodynamic volume of the caseinomacropeptide may be as high as 6 ml per gram of dry protein is taken into account.

There are fairly large differences in viscosity among lots of milk, and the hydrodynamic volume of the casein micelles must be an important variable. Content of calcium phophate in the micelles and $Ca^{2+}$ activity play a part. See (Section 13.1.) Increasing the pH of milk increases its viscosity (perhaps by swelling of casein micelles, and slightly decreasing the pH usually leads to a little decrease. A more drastic pH decrease causes the viscosity to increase, which is caused by aggregation of casein.

Decreasing the temperature of milk causes an increase in viscosity. The $\eta_o$ increases, but a more important variable is the hydrodynamic volume of the casein micelles. Moreover, some casein, particularly $\beta$-casein, starts to dissociate from the micelles, and dissolved casein molecules have a high hydrodynamic volume. Consequently, the viscosity rises, but the behavior is no longer Newtonian. See Figure 16.1C for the effect of temperature. More information is in the appendix, Table A.22.

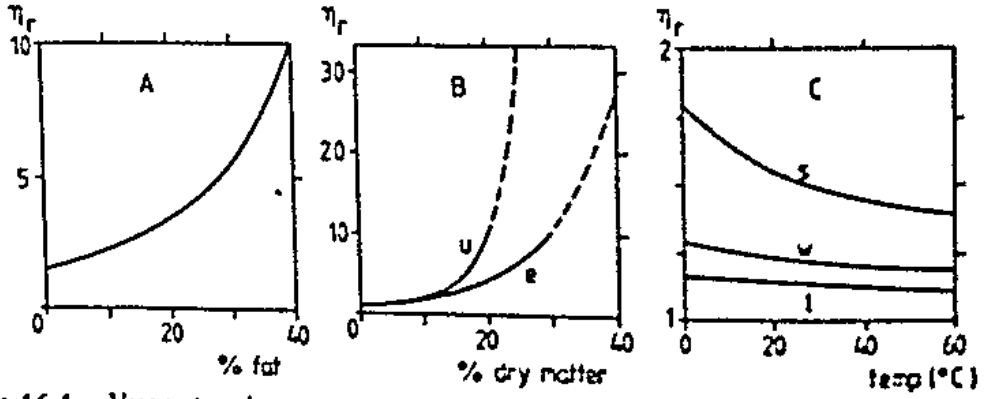



**Figure 16 1.** Viscosity relative to water ($\eta_r$) of milk products (A) Milk and cream, 40 C, (B) skim milk concentrated by evaporation (e) and ultrafiltration (u) (C) skim milk (s), whey (w), and 5% lactose (l) at various temperatures The broken lines indicate apparent viscosity (at high shear rate) After various sources

Heating of skim milk to such a degree that most of the whey protein is heat denatured causes an increase in viscosity by about 10% Homogenization has little effect, $\phi_r$ increases (because of the larger surface covered with protein), but $\phi_c + \phi_w$ decreases (by providing material for the surface layers)

## 16.2. NON-NEWTONIAN LIQUIDS

For many dispersions the measured viscosity decreases with increasing shear rate, this is called shear thinning Such behavior implies that the liquid has not a true but an apparent viscosity ($\eta'$), to be unequivocal we must state the shear rate at which it was measured The apparent viscosities are always higher than the viscosity as predicted by equation 16 1 The primary cause is aggregation of particles, the aggregates contain interstitial liquid and have irregular shapes, and consequently $\phi$ is increased effectively The aggregates are held together by interaction forces between the particles The shearing action of the flow exerts a stress (magnitude $\sim \eta_0 G$) on the aggregates, which may disrupt them into smaller and more rounded ones In this way effective $\phi$ and thus $\eta'$ decrease with increasing $G$ If the force acting on the aggregates is much larger than the interaction forces, the latter are negligible, and the liquid shows Newtonian behavior Consequently, the higher the shear rate, the smaller its effect on viscosity (But at very high shear rate, turbulence may occur, causing a seeming increase in viscosity )

There are always interaction forces between particles in a dispersion, but they are often too weak to become noticeable in the measurement of viscosity, because the shear rate is too high A $G$ of less than 10 $s^{-1}$ cannot be obtained in most instruments, and still lower shear rates may well give non-Newtonian behavior The sum of the interaction forces of the whole dispersion is determinant Hence, at higher $\phi$, deviation from Newtonian

flow persists up to a higher $G$; this also results from the stronger dependence of $\eta$ on $\phi$ at higher $\phi$. For instance, cream of 40% fat is (at 40°C) Newtonian for $G$ greater than 10 $s^{-1}$. In a certain cream of 49% fat viscosity was almost Newtonian near $G$ equal to 100 $s^{-1}$, with $\eta$ about 28 mPa·s; but at 10 $s^{-1}$, $\eta'$ had increased to 54 and at 0.7 $s^{-1}$ to 140 mPa·s.

Raw or low-pasteurized milk and cream usually show marked non-Newtonian behavior at low temperatures, because of the cold agglutination of the fat globules. (See Section 14.3.) Figure 16.2A gives examples. The lower the temperature, the stronger the forces between the globules, the higher the shear rate needed for Newtonian behavior, and the higher the apparent viscosity, also if corrected for the effect of temperature on $\eta_0$ and $\phi_c$. Even in cold raw skim milk or whey, the flocculation of agglutinin itself causes a little increase in $\eta'$.

As discussed, the shearing action of the flow breaks bonds between the particles. The bonds may reform; if they do so within a time shorter than that of the experiment, $\eta'$ depends on shear rate only, because for any value of $G$ a certain size of aggregates results, which decreases with increasing $G$. But if the time needed for breaking or reforming the bonds is longer, $\eta'$ depends on shearing history. This is true for cold agglutination; the bonds break slowly, hence $\eta'$ keeps decreasing for a while during shearing, and though $\eta'$ increases after stopping the shearing action, it usually does not come back to its original value.

Such a hysteresis of $\eta'$ versus shear rate is also conspicuous in cream containing homogenization clusters (Section 14.4.3) as shown in Figure 16.2B. Here, many of the bonds that are broken are not reformed, and after shearing once at a high rate there is little hysteresis.

Coalescence of fat globules causes little change in $\eta$, because $\phi$ does not change. But partial coalescence causes an increase; the granules formed enclose plasma and have irregular shapes. When the viscosity of heavy cream is measured, shear thickening may be observed, as the shear itself induces

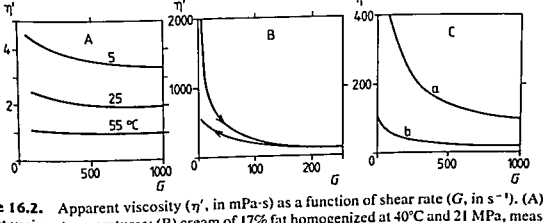



**Figure 16.2.** Apparent viscosity ($\eta'$, in mPa·s) as a function of shear rate ($G$, in $s^{-1}$). (A) Raw milk at various temperatures; (B) cream of 17% fat homogenized at 40°C and 21 MPa, measuring sequence first from low to high, then back to low $G$ (viscosity before homogenization was about 10 mPa·s); (C) retentate of ultrafiltered skim milk, protein content 16%, heated for 30 min at 95°C before (b) or after (a) ultrafiltration. Approximate examples after various sources.

partial coalescence Partial coalescence also appears to be the cause of the rebodying of cream by temperature fluctuation (See Section 14 2 2 ) In cream of 20% fat, $\eta'$ may double, and for 30% fat or more the cream may become very thick and plastic

Heating of milk causes some increase in viscosity, as stated above, because of a slight protein aggregation If the milk is first concentrated and subsequently heated, the increase in (apparent) viscosity is much larger than for milk concentrated after heating This is illustrated in Figure 16 2C, which also shows the marked shear thinning in ultrafiltration retentates of high protein content Whey concentrated by ultrafiltration may form a gel on heating The increase in viscosity and the eventual gelation of evaporated and sweetened condensed milks on holding also result from aggregation of casein micelles, though in the first stages an increase in the hydrodynamic volume of the micelles also may contribute

Solutions of polymers or macromolecules also show non Newtonian behavior, even if they are dilute The explanation is rather intricate, but at higher concentration mutual hindrance of the molecules is an important causative factor Apparent viscosity steeply increases with concentration Such effects are noticeable in concentrated whey, presumably because of the caseinomacropeptide (See also Figure 17 11 for Na caseinate solutions ) Often, polymers are added to milk products, mostly polysaccharides like alginates, pectins, locust bean gum, carrageenans, or carboxy methyl cellulose, they usually are called gums, thickening agents, or stabilizers Little polymer is needed to increase the apparent viscosity, because the polymers usually have a high molecular weight, and the increase in $\eta$ per unit mass of polymer is proportional to, for instance, $MW^{0.6}$ (This is very different from dispersions, where only the concentration and not the size of the particles affects viscosity ) Many bacteria produce a slime in the form of their glycocalix, which consists of fairly long polysaccharide chains anchored to the cell wall and protruding into the surrounding liquid Some strains of lactic acid bacteria (e g , of *Streptococcus lactis* and *Lactobacillus bulgaricus*) produce so much polysaccharide that they considerably increase the viscosity of sour milk Probably binding of casein micelles to the glycocalix significantly increases effective volume fraction, hence $\eta'$ Bacterial polysaccharides also can be isolated and used as thickening agents in milk products, examples are dextran and xanthan gums

## 16.3. GELS

When particles aggregate to such an extent that they form a continuous network throughout the liquid a gel is formed Like a solid, a gel has elastic properties, when it is deformed by stress, it recovers its original shape after removal of the stress, as the deformation has left the bonds between the particles intact But a gel also has viscous properties, because part of the

deformation is not recovered at removal of the stress; in other words, the gel flows when stressed as bonds are broken and new bonds formed, as in a liquid. If the stress is applied for a very short time, the elastic (i.e., recoverable) deformation is always predominant; if the stress lasts long, the viscous deformation (i.e., flow) is most conspicuous.

A gel thus shows viscoelastic behavior, which can be characterized by two rheological parameters, the elastic or storage modulus ($G'$) and the viscous or loss modulus ($G''$). If $G'$ is much greater than $G''$ the elastic properties prevail, if $G'$ is much less than $G''$ the viscous properties prevail. The overall resistance to deformation is expressed in the combined modulus $G^*$. The moduli give the ratio of the stress to the relative deformation and thus have the dimension of stress (e.g., $N \cdot m^{-2}$ or Pa); they depend, often strongly, on the time scale of the deformation.

The reasoning given above only holds when the deformation is (about) proportional to the stress (so-called linear behavior). Gels, unlike solids, often can be deformed considerably (e.g., by 10%) and still show linear behavior. At larger deformations this relationship breaks down, as bonds in the network are broken that do not reform within the time scale of the experiment. A still larger deformation soon causes yielding or breaking of the gel, as the network is locally destroyed. The stress needed to do this may be called a yield stress; it is not a well-defined quantity as it depends much on conditions, like geometry of the measuring instrument and time scale. Once deforming a gel to such an extent that some bonds are permenantly broken will alter its rheological properties.

The firmness of a gel as perceived in common practice is more related to a yield stress than to the modulus, and most instruments for testing gel strength determine a kind of yield stress (e.g., the force needed to push a probe into the gel). Yield stress and modulus are correlated naturally, but the correlation is by no means perfect, and some variables even may increase the modulus while decreasing yield stress. Probably, inhomogeneity of the gel is important; if its network has a limited number of weak strands this will affect greatly (i.e., diminish) the yield stress and leave the modulus almost the same.

Several gels can be made from milk products. The fat globules in cream may form a continuous network, for instance, by cold agglutination, thus causing a (weak) gel to form. We will consider predominantly casein gels. Casein micelles as such may be considered as very small regions with a gel-like character. The whole liquid becomes a gel as the casein micelles aggregate, by rennet action (forming curd, Section 13.3.4) or by acidification (as in yogurt and other sour milk products). Rennet and acid milk gels are rather similar in properties) though there are quantitative differences. The modulus of acid gels is higher. (See Figure 16.3). For very short deformation times the viscoelastic behavior is similar, but for longer times a rennet gel has relatively more the character of a liquid than has an acid gel. A piece of rennet gel thus easily flows under its own weight (also because of its

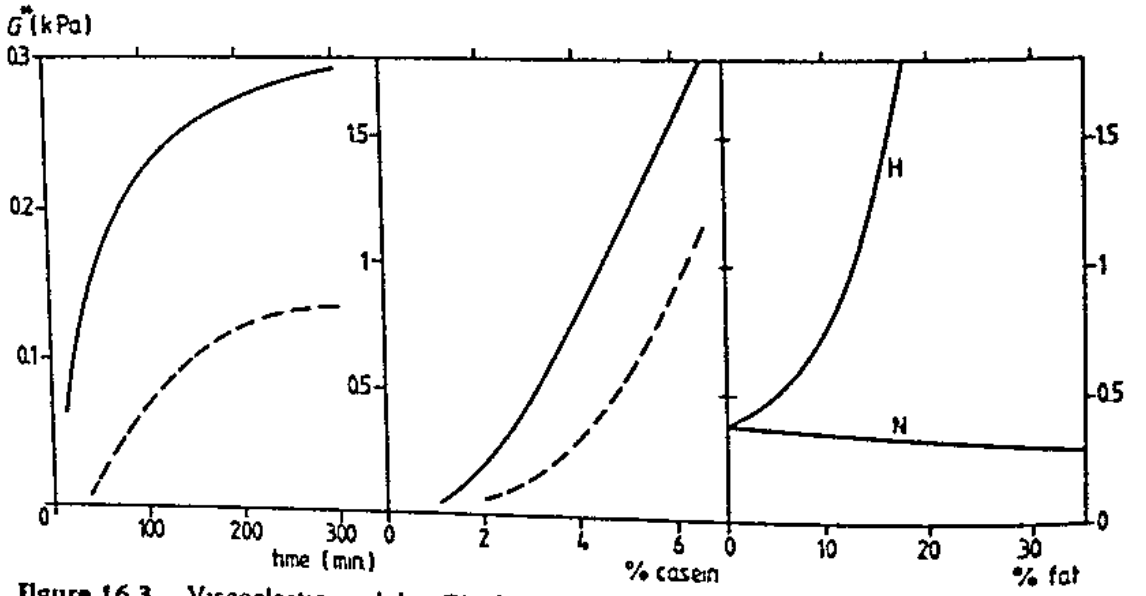



**Figure 16 3.** Viscoelastic modulus $G^*$ of acid (— — —) and rennet (———) milk gels at 30°C Variables are time after acidification or renneting, casein content varied by mixing ultrafiltrate and retentate, fat content varied by mixing skim milk with natural (N) or homogenized (H) cream Approximate examples after T van Vliet (unpublished)

lower modulus), while a piece of acid gel keeps its shape better An acid gel also breaks (yields) at a smaller deformation (it is "shorter")

Many factors influence the modulus and the firmness of milk gels

1. The time elapsed after aggregation has started (See Figure 16 3 ) Acid gels need about 24 h, rennet gels about 6 h (at 30°C) to obtain a final value of the modulus These changes are paralleled by changes in microstructure Directly after aggregation, the individual (para)casein micelles still can be distinguished in the network, but after a while they seem to fuse together, and the network now consists of fairly irregular strands without much internal structure The yield stress of a rennet gel reaches a plateau value after a much shorter time (See Figure 13 5 )
2. Temperature A gel made at a higher temperature is firmer But the effect of the temperature of measurement is the reverse, the gel becoming much firmer when it is colder
3. Casein concentration The effect is strong (Figure 16 3), as is only to be expected It makes a difference how the higher concentrations are obtained, by evaporation or by ultrafiltration, or by syneresis after the gel has been formed
4. A higher content of calcium phosphate in the micelles This gives (under otherwise the same conditions, e g , pH) a firmer gel
5. A lower pH This gives (down to the isoelectric pH of casein a firmer gel

6. Preheating the milk with such intensity that whey proteins become denatured. This causes a rennet gel to be weaker, an acid gel to be firmer.
7. The complicated effect of fat content. Natural fat globules tend to make the gel somewhat weaker. (See Figure 16.3.) They interrupt the network and thus make weak spots in the gel. But if the milk (or cream) has been homogenized, the fat globules become partly covered with casein and now participate in forming the network. Consequently, the gel can become much firmer. A practical example is sour cream, the firmness of which greatly depends on proper homogenization. The actual change in consistency depends on many factors, like preheating, homogenization temperature and pressure, type of measurement, and type of gel. A rennet gel may even become weaker (when measured as yield stress) from homogenization of the milk.

Another difference between acid and rennet milk gels is in their tendency to show syneresis. Rennet gels always do this. Acid gels that are kept still during acidification and for several hours afterwards do not show syneresis. Agitation during setting (gel formation and firming up) causes local breaking of the gel (when it is still weak) and syneresis of the pieces of gel remaining. In this way a viscous liquid is formed. Also, stirring of soured milk after setting causes breakup of the gel, and a viscous sour milk is obtained. The increase in viscosity over unsoured milk is caused largely by the presence of remnants of the network (or pieces of gel) that make up a high volume fraction. The more intense the stirring and the weaker the original gel (see points 1–7 above), the smaller the remnants and the lower the viscosity. The viscosity of sour milk is thus highly non-Newtonian.

Solutions of macromolecules also can gel. There are two types of gel. In one, intermolecular covalent bonds are formed, as in boiled eggwhite. Something like this happens when concentrated milk or concentrated whey is heated, because a few –S–S– bridges are involved in the gelation; still, these gels have largely the character of a network of aggregated particles. In the other types, the macromolecular chains form weaker bonds by a kind of local crystallization or by local helix formation, as in gelatin. There is usually a fairly sharp temperature below which a gel forms. Some thickening agents added to milk products, like gelatin, carrageenans, and alginates, do this if present in high enough concentrations. The interaction between $\kappa$-carrageenan and casein micelles mentioned in Section 13.2 is a special case. This gives milk containing very little $\kappa$-carrageenan viscoelastic properties, though with a very low yield stress. Pouring the milk out of a beaker is sufficient to disrupt the network, but it rapidly forms again. The weak network effectively prevents creaming or sedimentation (say, of cocoa particles in chocolate milk), and only some 0.02% of $\kappa$-carrageenan is needed as compared to, say, 0.15% for thickening agents that do not interact with the casein micelles in forming a network.

## 16.4. MILK FAT

Partially solid milk fat has a plastic consistency It is a viscoelastic material, but in common parlance we would call it a solid rather than a gel At very small deformations (e g $< 1\%$) the behavior is almost purely elastic, the elastic modulus being, for instance, 1 MPa At larger stresses, the material begins to flow, for instance, as depicted in Figure 16 4A At still larger stress, the relation is often roughly

$$\dot{D} = (\tau - \tau_B)/\eta_B \qquad (16\ 2)$$

where $\dot{D}$ is deformation rate or flow rate, and $\tau$ is stress The extrapolated or Bingham yield stress $\tau_B$ may be taken as a measure of consistency or firmness $\eta_B$ is the pseudo or Bingham viscosity The results of experiments as depicted in Figure 16 4 depend, however, on experimental conditions, like geometrical factors and time scale

The most important factors determining firmness are as follows

1. Because of Van der Waals attraction and the absence of electrostatic or steric repulsion, the fat crystals flocculate They form a network with meshes about one or a few micrometers in diameter At very small deformations, this network causes the elasticity of the fat At larger deformation bonds in the network are broken, and the force needed to do this is the largest contribution to the firmness of a freshly crystallized fat
2. Fraction of fat solid (Figure 16 4B) is important because it determines the number and to some extent the strength of the bonds in the network of crystals There is thus a large effect of temperature and fat composition (See Section 5 7 )
3. Size and shape of the crystals again mainly through point 1 above, also are important A milk fat with 20% crystals consisting of large

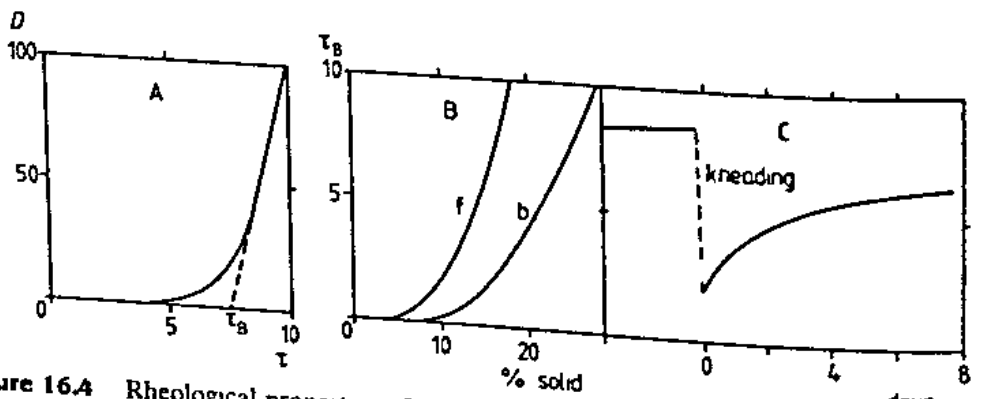



**Figure 16.4** Rheological properties of milk fat (A) Deformation rate ($\dot{D}$ s $^{-1}$) of milk fat as a function of stress ($\tau$ MPa) (B) firmness ($\tau_B$ MPa) as a function of percentage of fat solid where f pertains to milk fat b to butter (C) firmness as a function of time after kneading the fat Only meant to illustrate trends After various sources

spherulites is pourable; 10% solid fat in the form of small crystals may give the fat a solid appearance and a fairly high yield stress.

4. As discussed in Section 5.7, fat crystals may keep growing for a long time, even when the solid–liquid ratio does not increase significantly. Flocculated crystals then become sintered, and the network mentioned may grow into a solid structure, extending throughout the volume. During deformation this structure must be broken locally, which implies a greater firmness. Moreover, strong deformation, working, or kneading causes the firmness measured afterward to decrease, a phenomenon called work softening. Part of the lost firmness is regained slowly, at first because the crystals flocculate, then because of some sintering. These changes in firmness are illustrated in Figure 16.4C.

Another important consideration is that the structure of crystals and liquid fat never will be the same throughout the whole mass. Consequently, the strengths differ among regions. During deformation, particularly the regions with weak bonds will flow; hence, deformation is unevenly distributed.

Butter has an even more uneven structure. It contains moisture droplets that may weaken the structure slightly. Of particular importance is tbe fact that it contains intact fat globules, which hold part of the crystalline fat. The fat crystals inside globules cannot form networks with those outside. Consequently, for the same solid–liquid ratio, butter will have a less developed crystal network than milk fat; hence, it is less firm, as illustrated in Figure 16.4B.

The difference in rheological properties between a 50%-fat cream and a low-fat water-in-oil, butterlike product with about the same composition is striking; they may differ by a factor of $10^6$ in apparent viscosity. In the cream, the fat crystals cannot form structures throughout the volume; in the low-fat butter they can, and this is just the explanation. As soon as the cream is subject to partial coalescence, its rheological properties become closer to that of butter.

## *Suggested Literature*

An elementary discussion on rheological principles is by
G. W. Scott Blair, *Introductory Rheology* (New York: Academic Press, 1969).
An extensive and rigorous discussion of methods of measurement is in
R. W. Whorlow, *Rheological Techniques* (Chichester: Horwood, 1980).
Several aspects of rheology of foods are treated in
P. Sherman, *Industrial Rheology* (New York: Academic Press, 1970).
Some of these aspects also are found in Chapters II–IV of
ChoKyun Rha, ed., *Theory, Determination and Control of Physical Properties of Food Materials* (Dordrecht: Reidel, 1975).

Many aspects of gels are in

*Gels and Gelling Processes* Faraday Disc 75, 1974

Rheology of emulsions is discussed by

P Sherman ed , *Emulsion Science* (New York Academic Press, 1968), Chapter 4

Rheology of cream and butter are discussed by

H Mulder and P Walstra *The Milk Fat Globule* (Wageningen Pudoc, 1974)

# 17

# Properties of Milk Concentrates

Milk and milk products can be concentrated by removing water. This is applied to increase the lifetime of the concentrated product and to diminish its volume. Water can be removed by evaporation or by freezing; in the latter case the ice usually remains in the product. Evaporation to such a low water content that the product becomes solid-like usually is called drying, but we often will use the term *concentrated milk* (or *skim milk* or *whey*), regardless of the concentration factor.

Concentration also can be achieved by nonequilibrium processes like reverse osmosis, or by addition of dry components (e.g., adding milk powder to milk). A different kind of concentration is achieved by ultrafiltration, because besides water most soluble components are removed; mostly (e.g., Sections 17.2–17.5) we will consider only removal of water.

## 17.1. WATER RELATIONS

The paramount change to be considered is that of the water concentration. This implies changes in the reactivity of water and in its interactions with other components. This section gives an introduction to these aspects.

### 17.1.1. Water Activity and Sorption Isotherms

The chemical reactivity of a compound in a particular environment is determined by its thermodynamic activity. In many cases the activity is nearly equal to the concentration, but there are notable exceptions (e.g., ionic

substances, Section 4 2 1) In most food products water activity differs from its concentration Water activity is commonly expressed as a fraction, pure water having $a_w$ of 1 For ideal thermodynamic behavior, the so defined water activity of a solution is equal to the mole fraction of water (Raoult's law)

$$a_w \text{ (solution)} = \frac{\text{moles of water}}{\text{moles of water} + \text{moles of solute}} \qquad (17\ 1)$$

But, as mentioned, this is usually not true, $a_w$ being lower than predicted by the equation Bringing the solution into equilibrium with air, $a_w$ in product and air are equal Thermodynamic behavior of water vapor in air is ideal at the temperatures considered, so we have simply

$$a_w \text{ (air)} = \frac{p_w}{p_{w\,sat}} = \text{RH} \qquad (17\ 2)$$

where $p_w$ is water vapor pressure, and the subscript *sat* refers to air saturated with water, thus $a_w$ equals relative humidity (RH), which can be measured easily Consequently, $a_w$ of the product is known, it also is called equilibrium relative humidity (ERH)

Why does equation 17 1 usually not hold exactly? A substance that dissociates causes a higher depression of $a_w$ because its effective molar concentration is higher The same holds true for macromolecules that continuously change their conformation in solution Substances may be hydrated or bind water, thus effectively removing water molecules from the solution Considering milk components, it is clear that fat globules do not affect $a_w$ materially, for the same ratio of water to solids not fat $a_w$ will be the same, regardless of fat content The salts dissociate but mostly do not bind much water Sugars bind more water The proteins bind about 0 2 g of water per gram of protein under conditions as in milk

Any product has its characteristic relation between $a_w$ and water content for every temperature Such a relation is called water vapor isotherm or sorption isotherm The term *sorption isotherm* stems from the consideration that a comparatively dry product (say, casein or milk powder) will sorb water onto its surface or into its particles when brought in contact with moist air, for liquid products the term is less appropriate An example of a sorption isotherm is given in Figure 17 1 Most such curves depend somewhat on whether they are obtained by successively drying a wet product (desorption isotherm) or by letting a dry product take up water (resorption isotherm), for the same $a_w$ the desorption isotherm shows a higher water content This hysteresis must result from lack of equilibrium It often takes a long time (some days) after bringing a product in contact with air of a certain $a_w$ before its water content ceases to change perceptibly, particularly the final traces of water may be very difficult to remove Even after no further changes in water content can be detected hysteresis of de and resorption curves is observed but the effect is rather small for concentrated milk products

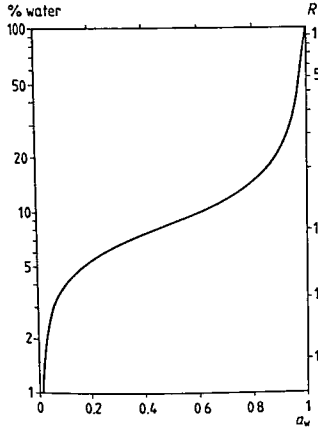



**Figure 17.1.** Approximate relation between water activity ($a_w$) and percentage of water in concentrated skim milk at room temperature. $R$ is the concentration factor (equation 17.3).

There is, however, a complication caused by the physical state of the lactose. In spray-dried milk powder, lactose is usually present in amorphous form. This implies that it is a highly concentrated solution with a comparatively small mole fraction of water; consequently, $a_w$ is fairly low. When lactose crystallizes (as $\alpha$-hydrate, Section 3.2.3) water of crystallization is taken up. This implies that equimolar quantities of lactose and water are removed from the solution. It can be derived from equation 17.1 that this results in a decrease in $a_w$ if $a_w < 0.5$, and in an increase if $a_w > 0.5$, for constant water content. Though equation 17.1 does not hold precisely, the trends are as predicted, as is shown in Figure 17.2A. Considerable hysteresis of sorption isotherms thus may occur. If milk powder (with amorphous lactose) gradually takes up water, lactose starts to crystallize at a certain stage (at about $a_w = 0.4$), and this alters the isotherm, so that in subsequent desorption a different curve is found. Some methods for determining water content in dry milk products give results exclusive of the water of crystallization. If water content is so defined, $a_w$ is higher for the same water content over the whole range. (See Figure 17.2A.)

The sorption curves and the hysteresis between them depend on temperature, partly because at higher temperature lactose will start to crystallize at a lower $a_w$, partly because $a_w$ shifts to higher values when increasing the temperature. The latter is illustrated in Figure 17.2B. The difference stems from the fact that it costs energy (in excess of the enthalpy of evaporation) to remove water from the product. The curves virtually coincide for $a_w >$ 0.8, so that removal of water down to that $a_w$ costs hardly any excess energy. The lower $a_w$, the larger the relative dependence of $a_w$ on temperature, and

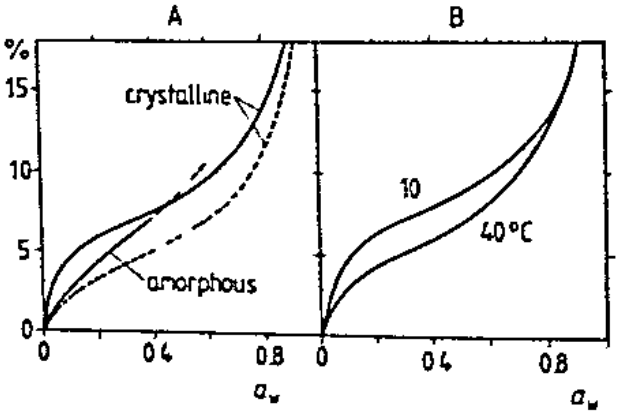



**Figure 17.2.** Sorption isotherms (percentage water in product against $a_w$) of skim milk powder (A) Desorption isotherm with lactose in the crystalline state and resorption isotherm with lactose in the amorphous state, the dotted line drawn for water content exclusive of water of crystallization, (B) desorption isotherms at two temperatures Approximate examples after various sources

the more energy it costs to remove water. To put it differently, the last water in a product is the most tightly bound

## 17.1.2. Water Binding and Holding

A water molecule that is bound to a surface or to some other molecule in the solution has lost part of its freedom of motion The residence time (i e , the time during which it stays at the binding site) is thus longer than the time during which a free water molecule stays at its place ($\sim 10^{-13}$ s) The residence time may vary widely, and the longer it is, the more strongly the molecule is bound Water of crystallization is very strongly bound (as in $\alpha$-lactose hydrate), and the same holds true for a few water molecules in the interior of globular proteins Weaker bonds are caused by H bonding and interaction of the water dipole with other dipoles or with ionized groups; the residence time is, for instance, $10^{-6}$ s Water also can be bound, though weakly, to a hydrophobic surface or group The explanation is loosely that water molecules adjacent to a hydrophobic group are forced to assume a more or less ice-like configuration, and their residence time is increased This happens at some parts of the surface of protein molecules All in all, proteins may bind up to a few grams of water per 10 g of protein

To this point we have considered only water molecules bound to a certain site. But more water can be affected by macromolecules like proteins Water can be held or imbibed as in a sponge A good example is the casein micelle in milk It has a voluminosity of some 4 ml $g^{-1}$ of casein (or less when excluding the outermost layer), which amounts to about 3 3 g water held per gram of casein, while the casein binds, say, 0 2 g water per gram A macromolecule in solution with an extended (e g , random coil) conformation may even take more water with it while moving through the liquid, for

dissolved β-casein at 4°C it amounts to about 8 ml·g$^{-1}$. The explanation is that the removal of water necessitates deformation of the casein micelle or the coiled macromolecule, and this costs energy. Imbibed water may amount to large quantities. If milk is renneted to form a gel, all of its water is held in the pores of the gel. (To be sure, it is serum rather than pure water that is held.) It is thus of some importance to distinguish clearly between bound water (water of hydration) and held (or imbibition) water, though the difference may not be so sharp as stated above.

Some factors affect the quantity of water bound. It decreases with increasing temperature; this is reflected in the increase in $a_w$ when the temperature is raised. As the water content of a product is lower, less water is bound to macromolecules in the product. This is partly from more solute molecules being present that compete for water as a solvent. But solutes also may compete directly for binding sites, and many salts are known to compete with water for binding sites on proteins. For instance, pure isoelectric casein binds about 0.5 g water per gram at $a_w$ of about 0.99, while in milk it binds maybe 0.2 g or less. Otherwise, factors like ionic strength (if above a certain minimal level) and pH little affect water binding by proteins. It may make some difference whether the protein is in contact with water or with air.

All factors that affect the conformation of macromolecules will influence the quantity of imbibed water. The same holds true for factors affecting intermolecular bonds. In milk the voluminosity of casein micelles is of particular importance. It decreases with increasing Ca concentration and increasing temperature; on the other hand, increasing ionic strength by adding Na or K salts causes an increase in voluminosity; the colloidal phosphate content of the micelles is decreased. The effect of pH is complicated: When pH is lowered, voluminosity of the micelles at first decreases (change in protein conformation), then increases (loss of colloidal phosphate), and finally decreases again until the isoelectric pH is reached. Heat denaturation of the serum proteins causes a marked increase in the amount of water they hold from, say, 0.4–2.5 g water per gram of protein, but not of the water they bind. Part of the imbibed water can be removed by applying pressure, as in a layer of centrifugally sedimented casein micelles, or when pressing curd.

The water held is mainly important because it affects the viscosity and some other physical properties of a product. Moreover, it lowers $a_w$ to a slight extent because it costs energy to remove water from the swollen macromolecules. The binding of water has further consequences:

1. The water activity is lowered, as discussed before, and the activity of some solutes consequently may be increased.
2. Bound water does not freeze; some even take this as a definition of bound water. Much of the water in milk products does not freeze

readily because the solutes cause depression of the freezing point, but it is assumed generally that this effect is not significant below −30° or −40°C

3. Bound water is not available as a solvent, and the concentration of solutes is thus higher than it would be if no water were bound But the amount of water not available as a solvent (nonsolvent water) may be higher than bound water because of negative adsorption of solutes at a surface present (e g , that of proteins) Negative adsorption means that the solute molecules stay away from the surface In milk products the surface area of proteins is large, for instance, some $10^3$ $m^2$ $g^{-1}$ for casein, so the effect may be considerable Several solutes, notably sugars, exhibit negative adsorption Figure 17 3 shows that the amount of nonsolvent water generally increases with the molecular size of the solute The figure also gives a possible explanation for this observation based on steric exclusion Solute molecules larger than water molecules must stay further away from the surface (envisioning their location to be in their center of gravity), thus leaving a layer of water devoid of solute This explanation may be an oversimplification but serves to illustrate the point It also agrees with the observation that the amount of nonsolvent water depends on the type (MW) but not on the concentration of solute Nonsolvent water for lactose is some 0 55 g $g^{-1}$ casein, for smaller molecules (not adsorbed) it will be less, and for large molecules like whey proteins it may be higher The negative adsorption of solutes causes a further decrease in $a_w$ and a slight increase in osmotic pressure On the other hand, solutes that do adsorb onto the proteins (say, $Ca^{2+}$ or some free fatty acids) lower $a_w$ to a lesser degree and the apparent quantity of nonsolvent water is negative for them
4. Bound water is not available as a reactant but it has to be very tightly bound not to react at all Usually, only the rate of reactions involving water is slowed down, as discussed in Section 17 2 2

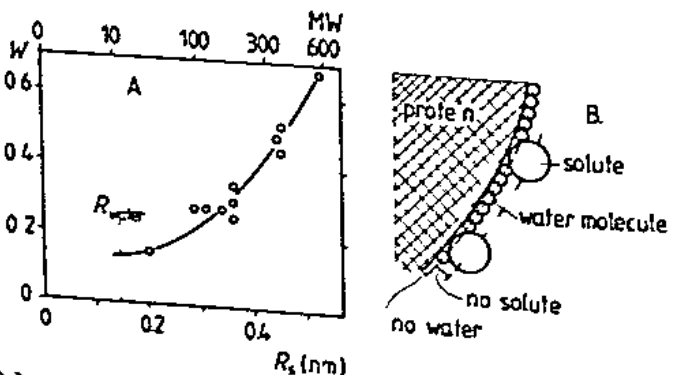



**Figure 17.3** (A) Nonsolvent water ($W$) in grams per gram of casein for different solutes as a function of molecular radius $R$ the molecular weight of the solute is also given (B) Visualization of negative adsorption of a solute by steric exclusion After various sources

It will be clear from the above that bound water is not a very precise and unequivocal conception. The amount depends on the criterion applied and on the method employed for determination.

## 17.2. EFFECTS OF CONCENTRATING

We define the concentration factor $R$ as the ratio of dry matter content (on a weight basis) of the concentrated product to that in the original material. Consequently, $1/R$ is the mass of the concentrated product divided by the mass before concentrating. For most effects of concentrating, the increase of the concentration of a substance relative to the amount of water is more important. This factor is given by

$$R^* = R(1 - c_i)/(1 - Rc_i) \tag{17.3}$$

where $c_i$ is the original dry matter content, expressed as mass fraction. $R^*$ becomes very large when $Rc_i$ approaches unity, that is, when almost all of the water has been removed.

### 17.2.1. Overview of Changes in Properties

Removal of water from milk causes numerous changes in properties, which generally are greater for higher $R$; several properties are about proportional to $R^*$. The changes depend to some extent on other conditions, such as heat treatment before, temperature during, and time elapsed after concentrating. Our knowledge about the effects of concentrating on milk is far from complete, particularly if much (say, more than half) of the water is removed. Important changes in properties caused by concentrating are:

1. The concentration of all components except water and a few volatiles increases. Therefore, the thermodynamic activity of solutes increases. The increase in activity is usually not proportional to $R^*$ because the activity coefficient changes. For most hydrophobic compounds and for sugars the activity coefficient increases.
2. The preceding item implies that the ionic strength increases, which causes the activity coefficients of ionic species to decrease (see Section 4.2.1), which, in turn, causes an increase in ionization and solubility of the salts. The thickness of the ionic double layer (the Debije–Hückel parameter $1/\kappa$—Sections 4.3 and 12.2.2) also is decreased.
3. Because of items 1 and 2 above the salt equilibria change. Ionization is decreased because of 1, but this is partly offset by 2. The $Ca^{2+}$ activity is increased only slightly, because calcium phosphate, which is saturated in milk, precipitates. Partly because of this precipitation,

the pH decreases by about 0 3 and 0 5 units for $R = 2$ and 3, respectively For $R = 2$ 5, the fraction of Ca that is in solution decreases from about 0 4 to 0 3 The ratio of monovalent to divalent cations markedly increases The isoelectric pH of the milk proteins decreases

4. Because of item 1 the colligative properties (osmotic pressure, freezing point depression, boiling point elevation) increase in magnitude The same holds true for electrical conductivity Because of item 3 the changes are somewhat less than proportional to $R^*$.
5. The conformation of proteins may change Item 3 above causes stronger internal salt bridges and a decreased electrical charge. Item 1 may imply a change in solvent quality All in all, the tendency of the protein molecules to attain a compact conformation probably is increased Their tendency to associate also increases
6. Casein micelles increase in size This is primarily caused by coalescence of micelles and not swelling The increase in size is less if the milk has been preheated to such an extent that most of the whey proteins have become associated with the casein
7. Dissolved substances become supersaturated and may precipitate Calcium phosphate has been mentioned, though an increase in the quantity associated with the casein micelles occurs instead of precipitation, the same holds true for tricalcium citrate Lactose becomes saturated in concentrated milks at room temperature when $R^* \approx 2$ 7 Moreover, the increase in activity coefficient mentioned in item 1 causes the solubility to decrease The solubility of lactose in sweetened condensed milk is only $\sim$ 10 g per 100 g water as compared to $\sim$ 20 g per 100 g in pure water at room temperature Most other substances (including the proteins) remain in solution, and not much precipitation occurs for $R^* < 12$
8. The water concentration decreases as does the water activity ($a_w$), as discussed in Section 17 1 An average relation between water content and activity of skim milk is in Figure 17 1 It is seen that a large proportion of the water must be removed before $a_w$ decreases appreciably Table 17 1 gives some examples of $a_w$ values of milk products
9. The hygroscopicity increases because the driving force for water uptake from the air is proportional to the difference between $a_w$ of the air and of the product The rate of water uptake depends, however, also on other largely unknown factors
10. The rheological properties are affected The viscosity increases (see, e g Figure 16 2) the liquid becomes non-Newtonian (shear thinning and viscoelastic), and finally solid like (say, at $R > 9$ for skim milk) Finally it becomes a fairly hard solid This all depends much on temperature

**Table 17.1. Approximate Water Activity ($a_w$) of Milk Products at Room Temperature**

| Product | Water Content % w/w | $a_w$ |
|---|---|---|
| Milk | 87 | 0.993 |
| Evaporated milk | 75 | 0.986 |
| Ice cream mix | 60 | 0.970 |
| Sweetened condensed milk | 27 | 0.830 |
| Skim milk powder (spray-dried) | 1.5 | 0.020 |
| Same | 3.0 | 0.100 |
| Same | 4.5 | 0.200 |
| Butter, unsalted | 16 | 0.990 |
| Butter, 2% salt | 16 | 0.920 |
| Cottage cheese | 79 | 0.988 |
| Most cheese varieties | 36–55 | 0.940–0.980 |
| Parmesan cheese | 30 | 0.920 |

**11.** The diffusion coefficient decreases. Figure 17.4 gives examples for the diffusion coefficient of water, and it is seen that the effect is very strong at low water content. For larger molecules, the dependence on water content is even stronger.

**12.** Most physical properties alter. For instance, density increases (Section 11.1), refractive index increases (Section 11.2), and heat conductivity decreases.

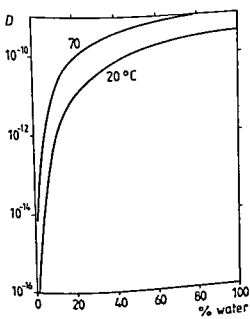



**Figure 17.4.** Dependence of the diffusion coefficient of water ($D$, in $m^2 \cdot s^{-1}$) in concentrated skim milk on water content at 20° and 70°C. After P. J. A. M. Kerkhof, unpublished.

## 17.2.2. Reaction Rates in Concentrated Products

The changes in properties may affect considerably the rates of reactions occurring in the concentrated products, and this is particularly important because such products usually are kept for a long time A problem in considering the effect of concentration on reaction rates is which factor is responsible, because so many conditions have been simultaneously altered If a single raw material, say, skim milk, is considered, it does not matter so much which variable (e g , water activity, percentage of water, or something else) is chosen, these variables are closely correlated But if we want to compare different products, say, concentrated milk and concentrated whey, uncertainty arises It is fairly customary to plot reaction rates against water activity, which often appears to be the most pertinent variable, but there are notable exceptions to this generalization

Chemical reactions of first order (or pseudo first order) usually decrease in rate when $a_w$ is decreased, log rate often varies approximately linearly with $a_w$ A good example is oxidative degradation of ascorbic acid, in many foods the rate decreases by about a factor $10^3$ when going from $a_w$ of 1 to $a_w$ of 0 The temperature dependence of the reaction ($Q_{10}$) also decreases with decreasing $a_w$ For reactions of higher order, the relation is often more complicated Because of increase in concentration of reactants (item 1 above), the rate at first increases when removing water If water is a reactant, its concentration is usually not rate determining at high $a_w$ but will become so at low $a_w$ At low water contents, the diffusion coefficients decrease (See Item 11 ) Moreover, other conditions, such as the pH, that affect the reaction may change Often, we find at first an increase, then a decrease in reaction rate, when lowering $a_w$

The Maillard reaction is an example of such behavior (Figure 17 5) Browning, loss of available lysine, and the development of off flavors result

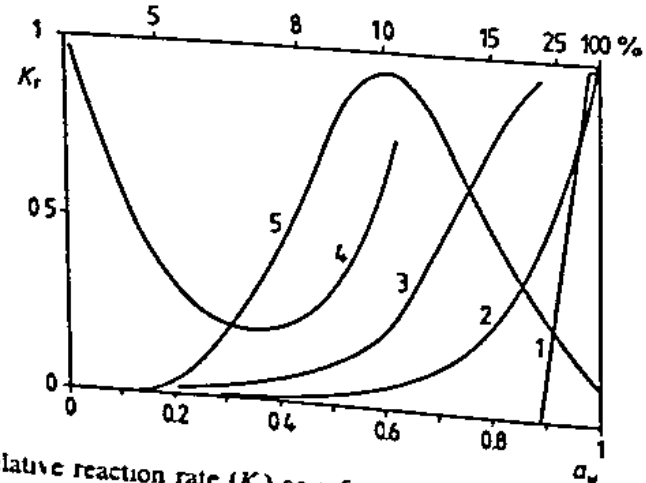



**Figure 17.5** Relative reaction rate ($K_r$) as a function of water activity ($a_w$) (1) Growth of *Staph aureus* (2) oxidative degradation of ascorbic acid (3) enzyme action (4) Lipid autoxidation (5) Maillard reaction Only meant to illustrate trends After various sources The upper abscissa scale gives the approximate water content (%) of concentrated skim milk

from Maillard reactions. (See Section 10.4.2.) The numerous chemical reactions involved will not depend in the same way on $R$ and thus the reaction pathway will also change. This may be one of the reasons why different authors have obtained fairly different results for the dependence of Maillard reactions on $a_w$. But the trend is always roughly as given in Figure 17.5.

The irreversible loss of solubility of milk protein also shows an optimum in $a_w$. It is a kind of age gelation (Section 13.4); in liquid products the viscosity increases before it comes to gelation; in milk powders the solubility as determined after mixing with water decreases. These reactions at first increase rapidly in rate with decreasing water content, but for $a_w < 0.6$ the rate decreases again. Consequently, concentrated milk products deteriorate rapidly at intermediate $a_w$, while they are much more stable at either high or low $a_w$.

Autoxidation of lipids (Section 5.6) follows a different pattern (Figure 17.5). Starting at very low $a_w$, the reaction rate decreases greatly with increasing $a_w$. Probable causes are that water lowers the lifetime of free radicals, interferes with the decomposition of hydroperoxides, and lowers the catalytic activity of metal ions.

Heat denaturation of proteins and, consequently, inactivation of enzymes and killing of microorganisms strongly depend on water content. Some examples are in Figure 17.6. Most reaction rates strongly decrease (by orders of magnitude) with increasing concentration, but the relations are rather variable. The rapid decrease in denaturation rate of chymosin at high water content can hardly be from a change in $a_w$, because $a_w$ varies so little with water content in this region. Following the discussion on heat denaturation in Section 10.2 (particularly equation 10.5), both $\Delta H^*$ (activation energy) and $\Delta S^*$ (activation entropy) decrease with decreasing $a_w$. Under most con-

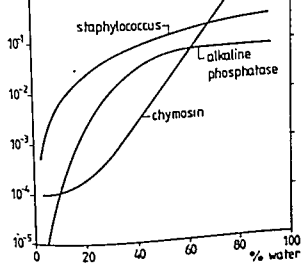



**Figure 17.6.** Reaction constants ($K$) for inactivation of enzymes and killing of a bacterium at 70°C as a function of water content. Inactivation of chymosin in concentrated whey, the others in concentrated skim milk. After A. L. H. Daemen, *Neth. Milk Dairy J.* 35 (1981):133.

ditions the decrease in $\Delta S^*$ is more prominent, and reaction rate decreases, but for some cases (none known as yet for milk products) the rate may increase again at still lower $A_w$ The decrease in $\Delta H^*$ implies that the dependence of denaturation rate on temperature also decreases with decreasing $a_w$, often strongly Some examples are given in Table 17 2 The rate usually depends not only on $a_w$ but on further composition as well, particularly for the killing of microorganisms This is not surprising, water removal will increase the concentration of any lethal or protecting substance present

All enzyme catalyzed reactions need water as a solvent and often as a reagent Consequently, lowering $a_w$ causes a decrease in reaction rate, though the precise relationship may differ Figure 17 5 gives an example, Lipolytic activity, though slight, has been observed in milk powder at $a_w \approx 0\ 2$

The growth and metabolic action of microorganisms closely depend on concentration, and it is generally assumed that $a_w$ is the prime variable The lag phase (time elapsing before the organisms added to the product start to multiply at an exponential rate) also may be longer at lower $a_w$ Many bacteria have an optimal $a_w$ for growth of about 0 99 and a minimal $a_w$ of 0 96–0 90 Figure 17 5 gives an example Small differences in $a_w$ thus can have considerable effect on growth rate Most yeasts have a minimal $a_w$ for growth of 0 91–0 87, most molds of 0 87–0 80 But there are organisms that can tolerate much lower water activities Halophilic bacteria may grow at an $a_w$ as low as 0 80–0 75, xerophilic molds to 0 75–0 65, and osmophilic yeasts to 0 65–0 60 The effects may differ, however, depending on which substances cause the low $a_w$ When water is removed, inhibiting and stimulatory substances also are concentrated

Crystallization of supersaturated water-soluble components also depends on $a_w$ If insufficient water is available as a solvent, the diffusion coefficient of the molecules to crystallize is so low that they cannot come to form nuclei, hence, crystallization is prevented The classical example is lactose in spray-dried milk powder, which is and remains present in amorphous form, unless the water activity is too high (say, $a_w > 0\ 4$)

Physical segregation of a concentrated product, like creaming of fat globules or sedimentation of lactose crystals, strongly depends on viscosity, hence on $R$ In milk powder kept at constant temperature and humidity, segregation cannot occur

When the temperature of a dried milk product is raised, reaction rates may increase because of the increase in $a_w$ (see Figure 17 2B) besides the temperature effect itself Consequently, a temperature rise easily may cause deterioration, for instance, insolubilization of protein and crystallization of lactose Moreover, differences in temperature throughout a homogeneous mass of product cause diffusion of water from the regions of high to those of low temperature, because water will always move toward the region of lowest $a_w$ Suppose that an airtight bag of milk powder is cooled temporarily from the outside The outer layer falls in $a_w$, sorbs water coming from the inner part of the bag and thus acquires a higher water content If the original

**Table 17.2. Kinetics of Inactivation of Alkaline Phosphatase and Killing of Some Bacteria by Heat Treatment in Skim Milk and Skim Milk Powder**

| Species | $a_w$ | $T^a$ (°C) | $\Delta G^*$ (kJ·mol$^{-1}$) | $\Delta H^*$ (kJ·mol$^{-1}$) | $\Delta S^*$ (J·mol$^{-1}$·K$^{-1}$) | $Q_{10}$ |
|---|---|---|---|---|---|---|
| Phosphatase | 0.99 | 63.0 | 100 | 432 | 990 | 100.0 |
| | 0.35 | 97.5 | 110 | 371 | 705 | 26.0 |
| *Staphylococcus* sp. | 0.99 | 64.0 | 99 | 426 | 970 | 90.0 |
| | 0.35 | 73.0 | 103 | 193 | 260 | 7.0 |
| *Serratia marcescens* | 0.99 | 45.0 | 95 | 496 | 1260 | 350.0 |
| | 0.35 | 50.5 | 97 | 152 | 170 | 5.7 |

*Source:* After A. L. H. Daemen, *Neth. Milk Dairy J.* 35 (1981):133.
[a]At this temperature the reaction constant $k^* = 0.0023\ s^{-1}$ ($D \approx 10^3$ s).

temperature is now restored, the outer layer has a higher $a_w$, which may cause deterioration before the lower equilibrium $a_w$ is reached again

## 17.3. EVAPORATION

Evaporation (i e , removal of water by boiling) is applied to obtain such products as evaporated milk and sweetened condensed milk, which have a fairly long lifetime and are convenient for certain uses Evaporation of milk, skim milk, and whey also is performed to save on costs of drying in making powdered products (removal of water by boiling is much cheaper than by spray drying) Evaporation always is done under reduced pressure, in order to reduce the temperature and thus minimize unfavorable reactions taking place during the process

The concentration has several consequences for product properties and stability, as discussed in Section 17 2 But evaporation generally is restricted to a fairly low $R$ (say, 5), so that $a_w$ usually is not below 0 97, hence the changes are not extensive Other changes resulting from boiling are effective removal of dissolved gases ($N_2$, $O_2$, $CO_2$), reduction of some off flavors caused by volatile components, and considerable damage to the fat globules, mainly splitting them into smaller ones and changing their surface layers The fat therefore may be subject to considerable lipolysis after evaporation, unless lipase has been inactivated properly

Limits to the degree of concentration by evaporation (say, to 55% dry matter in skim milk) stem mainly from the suitability of the concentrated milk for processing, the increased viscosity greatly hinders flow (hence, heat transfer), and fouling of heated surfaces (including those of the evaporator itself) becomes a problem Furthermore, the stability of the milk decreases with increasing concentration, Maillard reactions and the insolubilization of protein begin to occur at appreciable rates if $R$ is high, particularly at high temperatures One manifestation is a rapid increase in viscosity, eventually leading to gelation Consequently, a highly concentrated milk cannot be kept long Lactose may start to crystallize (Section 3 2) though this rarely happens in concentrated milk or skim milk, as considerable supersaturation is possible, in other words, nucleation is very slow In concentrated whey (where often $R^* > 20$) crystallization of lactose is certainly possible, and this is done on purpose, for instance, to separate lactose from the whey or to produce whey powder with crystalline lactose

*Evaporated milk* is concentrated mostly to $R = 2\,0$–$2\,5$, on further concentration the product is insufficiently heat stable But $a_w$ is not lower than about 0 98, and this allows rapid microbial spoilage, so that the product has to be sterilized Problems are heat stability (Section 13 5) and age gelation (Section 13 4) The product has a cooked flavor and slowly develops a stale flavor, along with brown discoloration If it is UHT-heated, the flavor is

better and the color whiter, but it nevertheless shows browning, and problems from action of heat-stable enzymes (lipases and proteinases) may occur. Homogenization of evaporated milk is needed to prevent rapid creaming, but it renders the product less stable to heat coagulation.

*Sweetened condensed milk* is concentrated much more ($R \approx 7$) and, moreover, sucrose is added. A typical composition is 8% fat, 20% milk solids-not-fat, 45% sucrose, and 27% water. The concentration of the sucrose solution is thus about 62.5% w/w (not considering nonsolvent water). The $a_w$ is about 0.83, so that microbial spoilage will be absent if contamination with osmophilic yeasts and micrococci is avoided and $O_2$ content is low enough to prevent growth of molds. The concentration is, however, not sufficient to prevent all enzyme action, and a fairly intense heat treatment (before concentrating) is needed to inactivate the enzymes. It is somewhat surprising that chemical deterioration is relatively slow. Presumably the sucrose has a protecting role. It lowers the $Ca^{2+}$ activity, but then $Ca^{2+}$ ions have little effect either on browning or on age gelation. Because viscosity is so high (say, 10 Pa·s), creaming is no great problem, but sedimentation of lactose crystals may be. Lactose is far supersaturated (by a factor of 4 or more), also because the high sucrose content considerably diminishes its solubility, and lactose crystals are formed. They should be very small (say, $< 8$ $\mu$m length) for sedimentation to be negligible and to avoid a product with a sandy mouthfeel; this implies a number of some $10^9$ crystals per milliliter. It can be achieved best by adding seed lactose before crystallization starts.

## 17.4. FREEZING

When milk is cooled it starts to freeze at about −0.54°C (if no supercooling occurs), and concentrated milk at a corresponding lower temperature. Pure ice is formed, and the milk solution thus becomes concentrated, thereby further decreasing the freezing point. The lower the temperature, the higher the proportion of the water that freezes (Figure 17.7) and the more concentrated the remaining solution. All of the consequences of concentrating mentioned in Section 17.2 apply. The water activity is a function of temperature only, if there is equilibrium among the three phases moist air, solution, and ice, because the vapor pressure of ice (hence its $a_w$) is a function of temperature only (for constant pressure). (The relation is given in Figure 17.7.) The changes in composition of the solution are considerable. At −8°C, for instance, the total solids content of the remaining liquid in frozen skim milk is about 53%, corresponding to $R^* \approx 12$. Making an ultrafiltrate of the liquid (at −8°C), it has a pH of about a unit lower than that of an ultrafiltrate of the original skim milk. The ionic strength may be almost ten-fold higher, the $Ca^{2+}$ activity is greatly increased, and the quantity of calcium phosphate in

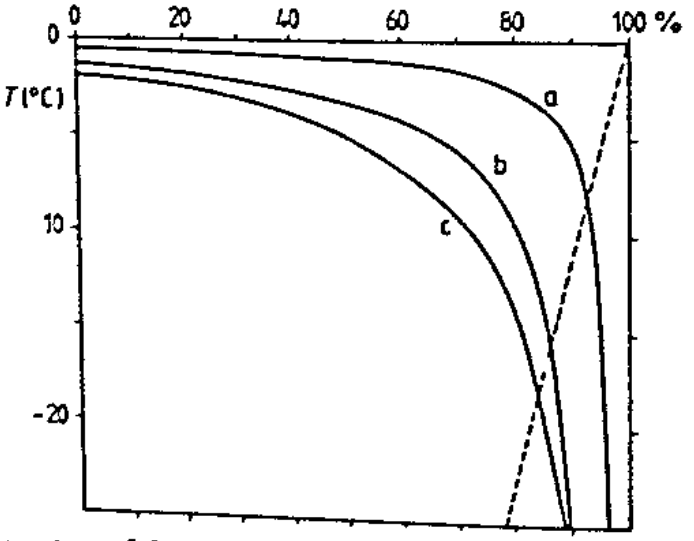



**Figure 17.7.** Percentage of the water frozen (at equilibrium) in milk products as a function of temperature (a) Skim milk, (b) concentrated skim milk ($R = 2.4$) (c) ice cream mix The broken line gives the water activity (%) in ice–water mixtures Approximate examples after various sources

the casein micelles also becomes higher, despite the decrease in pH At still lower temperature, other substances besides calcium phosphate start to precipitate

Lactose complicates this picture Figure 17 7 pertains to equilibrium conditions, and these are usually not attained because lactose is so easily supersaturated It becomes saturated near $-2°C$, but if milk is frozen quickly to below $-23°C$, it will not crystallize at all It is present in a dissolved state in a high concentration, causing considerable freezing point depression, preventing part of the water from freezing If the temperature is raised somewhat after freezing, or if freezing proceeds more slowly, most of the lactose may crystallize, this happens particularly in frozen concentrated milk Now, more ice is formed and the concentration of salts becomes much higher, with all of its consequences

In a frozen milk that is cold enough (e g , $< -20°C$) the temperature itself and the water activity are so low as to prevent microbial and enzymic spoilage Most chemical reactions proceed at negligible rates also, but autoxidation of lipids is still possible A problem may be insolubilization of the protein, this concerns aggregation of the casein micelles, and it becomes manifest after the product is thawed The freezing itself is not the cause Most probably, the casein is salted out, the aggregation is usually reversible, though redispersion occurs slowly The low pH, high ionic strength, high $Ca^{++}$ activity, and poor solvent quality of the liquid remaining unfrozen may be held responsible As long as the lactose remains amorphous, the casein usually keeps its stability, but crystallization of lactose causes fairly rapid aggregation of casein It is thus important to freeze quickly and keep the temperature below $-20$ C Addition of sugar (e g , sucrose) considerably improves the stability as it decreases the salt concentration, the explanation

is that at a given $a_w$ (determined by the temperature) more sugar implies more water (less ice), hence a lower ratio of salt to water. Hydrolysis of the lactose before freezing improves protein stability for the same reason; it increases molar sugar concentration and prevents or at least diminishes sugar crystallization. The stability is, of course, much less in frozen concentrated milk. Preheating the milk to such an extent that the whey proteins are denatured causes decreased stability, presumably because the whey proteins are now much more sensitive to $Ca^{2+}$ ions and participate in the aggregation.

Freezing causes the viscosity of the remaining liquid to increase and diffusion coefficients to decrease. At low temperature (e.g., −30°C) frozen milk is almost fully solid, and molecular transport is virtually impossible. During freezing of skim milk, considerable segregation occurs, ice crystals forming where the temperature is lowest, and concentrated liquid accumulating elsewhere (i.e., usually in the center). The segregation in crystals and concentrate, in principle, can be put to use in the freeze concentration of milk; the crystals are then separated from the concentrated milk, but the loss of dry matter adhering to the crystals is too high to make such a process economical. With increasing fat content and a smaller size of fat globules segregation is less, and homogenized cream changes on freezing into a fairly homogeneous mass. Freezing causes mechanical damage to the fat globules, the more so as the ice crystals are larger (slow-freezing) and the temperature is lower, so that subsequent thawing causes formation of fat granules or lenses. The fat emulsion remains stable if homogenized milk is frozen rapidly, but cream cannot be frozen without gross instability occurring.

## 17.5. DRYING

Drying is applied mainly to milk, skim milk, whey, sweet-cream buttermilk, and composite products like ice cream mix and infant foods. These liquids can be dried in various apparatus giving fairly different products, but spray drying is by far the most common, and we will concentrate on that.

Small droplets of milk (or concentrated milk, rather) are sprayed into hot air and change into dry powder particles within a second. Their water content is mostly about 3%, or 4% for skim milk powder. Variation in processing conditions may have a large effect on product properties. The main variables are (1) preheating of the milk, (2) degree of preconcentration, (3) manner of producing the droplets, either with a pressure nozzle or a spinning disk, (4) pressure and circumferential disk speed, respectively, and (5) temperatures of the air at the start and at the end of drying (related to inlet and outlet temperature, for instance, 200° and 80°C, respectively). This book is not the place to discuss these aspects.

The spray-dried particles are roughly spherical and about 5–100 $\mu$m in size, though greater variations occur if processing conditions are extreme. The volume-to-surface average particle diameter $d_{vs}$ (see Section 12.4) is

mostly 20–60 μm, and the size distribution is fairly wide, the relative standard deviation $\sigma_s$ being mostly 0 4–0 7 Particles may become agglomerated (i e , held together in aggregates comprising numerous individual spheres) Most particles contain vacuoles, during spraying small air bubbles are trapped in the droplets, and these bubbles expand in volume during drying The air pressure in the vacuoles is thus below atmospheric (mostly 0 2–0 5 atm) in freshly made milk powder, but they slowly fill with air (in several days in whole milk powder, in several weeks in skim milk powder) The volume of the vacuoles $V$ is 50–400 $cm^3\ kg^{-1}$ in most powders, nozzle spraying yields a smaller vacuole volume than does disk spraying, and $V$ mostly decreases with increasing concentration factor of the sprayed milk In most powders the vacuoles are fully inside the powder particles, but if the outlet temperature during drying is high, many vacuoles crack and come into contact with the surrounding air

Density of a powder can mean various things The true density $\rho_t$ may be defined as that of the material, excluding the vacuoles, $\rho_t$ is about 1300 kg $m^{-3}$ for dried whole milk, about 1480 kg $m^{-3}$ for dried skim milk, and about 1560 kg $m^{-3}$ for dried whey The particle density $\rho_p$ includes the vacuoles, and is thus given by

$$\rho_p = \frac{\rho_t}{(1 + V\rho_t)} \tag{17 4}$$

In skim milk powder $\rho_p$ is usually 900–1350 kg $m^{-3}$ The bulk density $\rho_b$ is the density of the powder, including air between the particles, its reciprocal is called the packed volume Bulk density is given by

$$\rho_b = \rho_p(1 - \epsilon) = \rho_t (1 - \epsilon)/(1 + V\rho_t) \tag{17 5}$$

where $\epsilon$ is the porosity (i e , the void fraction between particles) Generally, $\epsilon$ = 0 40–0 75, and the more sticky (or less free flowing) a powder is, the higher its porosity Whole milk powder is more sticky than skim milk powder and thus has a higher porosity The porosity decreases when a mass of powder in a container is vibrated or tapped somehow For whole milk powder $\epsilon$ may decrease, for instance, from 0 70 to 0 45 by tapping, for skim milk powder from 0 55 to 0 40 Consequently, $\rho_b$ depends much on the way of handling the powder, usually a compacted bulk density (i e , after tapping) is given All in all, $\rho_b$ can vary widely, for instance, from 300–800 kg $m^{-3}$

A milk powder particle consists of a continuous mass of amorphous lactose and other components of low molecular weight in which fat globules, casein micelles, and whey proteins (and usually vacuoles) are embedded The lactose remains amorphous because the time during which the lactose concentration is such that crystallization is possible (say, between $R^*$ = 10 and $R^*$ = 80) is far too short If the powder takes up water so that $a_w >$ 0 4, crystallization becomes possible This may cause considerable agglomeration, because the lactose crystals of adjacent powder particles become continuous The result in stored powder is caking, which means that the powder first forms hard lumps and finally turns into a continuous solid mass

The fat globules in milk powder are changed by the evaporation and the spraying process preceding drying. Considerable splitting of fat globules occurs, and they become partly covered with micellar casein and whey proteins. (See Section 14.4.2.) Some coalescence of fat globules occurs during drying. Often, a free fat content of whole milk powder is estimated; this is the amount of fat that can be extracted by an organic solvent. Such a procedure indeed removes any fat that is not present in globules, but as a thin layer at the surface of the powder particles. This surface fat is, however, only a small quantity (if present) and represents but a small fraction of the fat extracted. The remainder originates from fat globules that are in contact with the particle surface or with tiny cracks or pores that often exist in the powder particles. Consequently, the free fat content tells more about the structure of the particles than about the state of the fat.

The drying is so rapid that the stage during which proteins denature at a measurable rate is negligibly short. Under usual conditions, the temperature of the drying droplets does not reach a value above 70°C before they have lost almost all water. This implies that whey proteins remain soluble, most enzymes active, and all but the most heat-labile bacteria viable during spray drying; see also Figure 17.6 and Table 17.2. (The drying itself may kill some microorganisms, even at room temperature, but this varies greatly among species.) Conditions, however, may be more severe for some drying droplets, especially very large ones and those that by chance stay comparatively long in the dryer; this is more prominent for a high outlet temperature of the air. Such conditions may cause some powder particles to contain denatured whey proteins.

A small fraction of the powder may even be rendered insoluble in water (i.e., small proteinaceous lumps remain after dissolving the powder in water). This usually is expressed in the solubility index (clearly a misnomer, as it is a measure of the amount insoluble), the volume of the sediment remaining after dissolving the powder in water and centrifuging the liquid is determined. Besides small lumps of powder that sediment, others may cream because of their high fat content (the so-called flecks). The flecks predominantly consist of aggregated fat globules held together by gelled protein. The cause of the insolubilization seems to be similar to that of age gelation. (See Section 13.4). If milk powder is held for some months at a high water activity (say, $a_w$ 0.5) or high temperature (say 50°C), a substantial portion of the protein has become insoluble. Storage under proper conditions does not lead to important changes, even for a few years.

It is not so easy to prevent off-flavors from developing. When freshly made milk powder is reconstituted (i.e., dissolved in water to a dry matter content as in milk) it tastes almost like milk, that is to say, like the milk as it was just before drying, including any cooked or other off-flavors that it had acquired. Part of the volatile flavor components get lost during spray drying, but this is not important for most milk products. During storage of milk powder stale and tallowy flavors develop. The stale flavor probably results from Maillard reactions. (See Sections 10.4 and 18.4.1.) Its rate of

formation strongly increases with $a_w$ (Figure 17 5) and temperature Fat autoxidation is of little importance in skim milk powder but a major problem in whole milk powder. The rate at which the tallowy or oxidized flavor develops increases greatly with decreasing $a_w$ (See Figure 17 5 ) Consequently, a careful optimization of water content is needed to keep both Maillard and autoxidation reactions at an acceptable level The normal precautions should be taken to minimize fat autoxidation. (See Section 5 6 ) But if the milk has not received a fairly severe heat treatment before drying, the powder cannot be kept long from obtaining an oxidative flavor. Removal of $O_2$ from the powder by flushing with $N_2$ helps to alleviate oxidative deterioration But the presence of vacuoles containing $O_2$ make its effective removal difficult; to achieve it, the flushing with $N_2$ should be done twice, preferably with an interval of a few days to permit diffusion of $O_2$ out of vacuoles

Milk can be *roller dried* A thin film is dried on a heated drum and is scraped off and subsequently ground This leads to fairly large, flaky particles The heat treatment of the drying milk is severe, because drying takes a longer time and occurs at a higher temperature than in spray drying Consequently, whey proteins will be denatured, a substantial portion of the powder may be insoluble, and appreciable browning may have occurred Most of the fat globules have coalesced

*Freeze dried* milk powder has rather different properties Milk, preferably concentrated milk, is frozen quickly, and ice is sublimated under reduced pressure (10–100 Pa), hence low temperature (< 20 C), this removes most of the water, leaving a spongy mass of dried milk Residual water (a few percent) is removed by raising the temperature (e g , to 50°C) The freezing and drying causes considerable coalescence of fat globules, but chemical changes are slight A powder is obtained by gingerly grinding the porous mass The bulk density of the powder is relatively low, depending on preconcentration Roughly speaking, the volume of the product does not change after freezing Freeze drying of milk offers no real advantage over the much cheaper spray drying It is only used for some special products, such as lactic starters

Several types of *foam drying* have been applied, mostly for composite products The concentrated milk is made into a foam (with air or $N_2$) and dried at a low temperature to a voluminous mass, which is ground after drying. The powder obtained can have excellent flavor and solubility

## 17.6. MEMBRANE PROCESSES

In the membrane processes considered here, a solution is enclosed in a system with a semipermeable membrane and put under pressure The solvent (water) and part of the solutes pass the membrane (giving the permeate), part of the solutes do not and are thus concentrated in the retentate There

are also electrically driven membrane processes, such as electrodialysis; the latter is briefly mentioned in Section 4.5.

In *ultrafiltration* the membrane is effectively a filter through which small molecules can pass, while colloidal particles and macromolecules are retained. For milk this implies that globular proteins and larger particles do not pass; they include casein micelles, fat globules, and cells (the permeate is thus sterile). Ultrafiltration is applied to skim milk and whey (the fat usually is removed beforehand by centrifugal separation). The aim is, for instance, to produce a protein-rich product (e.g., from whey) or a step in an alternative way to make some types of cheese (removal of most of the soluble part of milk before renneting so that little or no syneresis is needed and most whey proteins are enclosed in the cheese).

Concentration by ultrafiltration differs greatly from concentration by evaporating water. Only some components are concentrated; in principle ionic strength and water activity remain constant, and protein conformation is almost unaltered. In fact, items 2–9 listed in Section 17.2.1 do not apply. Consequently, a retentate of skim milk exhibits comparatively little browning during storage or heating, and it is much more heat-stable than evaporated milk. Some changes in physical properties are considerable, and especially viscosity increases much more with concentration than in the case of evaporation. (See Figure 16.1.B.) The high viscosity of the retentate is usually the limiting factor in ultrafiltration; in the case of skim milk about 25% dry matter can be attained.

The previous paragraph is, however, an oversimplification to some extent, because smaller molecules may be partly retained. We define the reflection quotient of a solute as

$$Q_r = \frac{(q_w/c_w) - (q_s/c_s)}{(q_w/c_w)} = 1 - \frac{q_s c_w}{q_w c_s} \qquad (17.6)$$

where $q$ means flux through the membrane (quantity passing per unit time) and $c$ is concentration (at the pressure side), while subscripts w and s refer to water and solute, respectively. Ideally, $Q_r = 0$ for small molecules, and $Q_r = 1$ for proteins, but this does not happen. $Q_r$ gradually changes with molecular size (partly because of a spread in pore width in the membrane), and the relation between $Q_r$ and molecular weight depends on the type of membrane. (See Figure 17.8.) Moreover, $Q_r$ of small molecules increases to some extent with the pressure difference $\Delta p$. (The explanation is loosely that any retention of solute causes a difference in osmotic pressure on either side of the membrane $\Delta\Pi$. Water flux is now proportional to $\Delta p - \Delta\Pi$, solute flux to $\Delta p + \Delta\Pi$, which partly compensates for the retention. The compensation is relatively smaller for higher $\Delta p$.) A further complication is that the activities in the solution, rather than the total concentrations ($c$), are the relevant variables. Solutes may be adsorbed onto or otherwise associated with the proteins or particles retained, and some of the water will

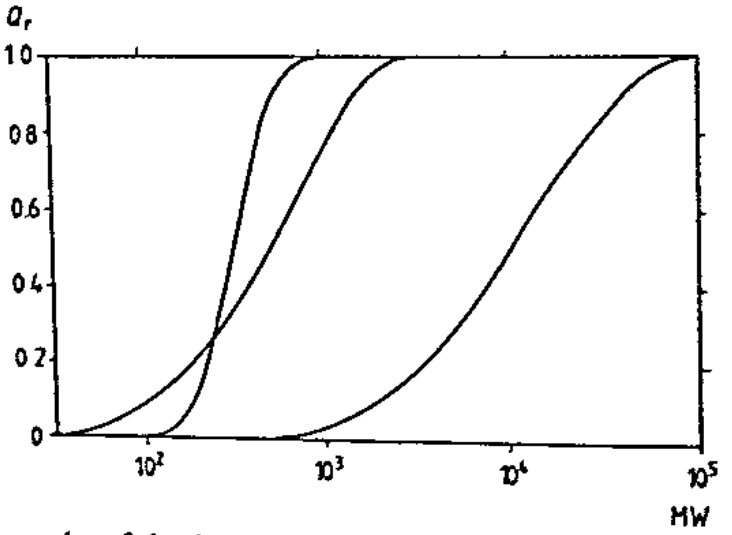



**Figure 17.8.** Examples of the dependence of the reflection quotient ($Q_r$) of three different membranes on molecular weight After various sources

not be available as a solvent (See Section 17 1 2 ) By taking these factors into account, "true" reflection quotients may be obtained

In the ultrafiltration of skim milk and whey, proteins or polypeptides of low molecular weight (e g , the proteose–peptones) partly pass the membrane ($Q_r < 1$) Usually, $Q_r$ is for lactose 0 02–0 15, for citrate 0 01–0 10, and for most smaller ions negligible Because the anions are on average larger than the cations, the pH of the permeate is 0 04–0 10 units higher than that of the original skim milk The composition of the retentate thus alters during ultrafiltration, its lactose and citrate contents slowly increasing, if expressed per kilogram of water (The concentrations expressed per kilogram of retentate increase less or may even decrease because of the increase in dry matter, especially protein ) Ionic strength of the retentate will increase somewhat, and its pH will decrease In practice, the retention ratio of a solute s is commonly used It is given by $1 - [s]_p/[s]_o$, where subscripts p and o refer to permeate and feed liquid, respectively, the concentrations should be expressed per kilogram of water Initially, the retention ratio equals $Q_r$ Because of the change in composition of the retentate during ultrafiltration, the permeate also will change and thereby the retention ratios, although the reflection quotients remain the same In one mode of operation, called diafiltration, the volume of retentate is kept constant by adding water, this process results in a considerable change in composition, which is reflected in the composition of the permeate

*Reverse osmosis* or hyperfiltration is applied to remove water, its prime advantage over evaporative concentration is saving energy It differs from ultrafiltration in the use of a different kind of membrane and in the application of a much higher pressure The membrane does not act so much as a filter but more as a material in which water can dissolve and thus pass through, while the solutes cannot All of the changes listed in Section 17 2 apply, but reverse osmosis is performed at lower temperatures than evaporation

High pressures are needed for this process. The driving force is not given by $\Delta p$ but by $\Delta p - \Pi$, where $\Pi$ is osmotic pressure of the retentate. For skim milk or whey, $\Pi$ is about 700 kPa, and it increases with concentration, though somewhat less than proportional with $R^*$. Usually, $\Delta p$ is 3–10 MPa, and the increase in $\Pi$ is usually the limiting factor for the concentration factor. Ideally, $Q_r$ is 1 for all solutes. In practice, $Q_r$ for small molecules is 0.75–0.99; $Q_r$ depends on type of membrane, increases with $\Delta p$, and decreases with increasing $R$. The permeate is thus by no means pure water; it contains some salts, organic acids, urea, and other small molecules. The loss of volatile substances from the concentrate is mostly much less than in evaporation.

## 17.7. PROTEIN PREPARATIONS

The milk proteins from skim milk, buttermilk, and whey can be concentrated in various ways. The concentrates usually are dried and are available in powdered form; Table 17.3 gives some important examples. These preparations are used in several food products. Nonfood uses once were extensive (e.g., casein for manufacture of plastics or for paper coating) but have much diminished.

As seen in Table 17.3, most preparations are far from pure; some are more like protein-rich food products. The degree to which other milk solids are removed is variable, according to isolation principle, intensity of washing, and so on. Moreover, the proteins may bind several substances, depending on conditions like pH, notably cations (counterions) and, in the case of casein and heat-denatured whey proteins, calcium phosphate. The "ash" also includes the ester phosphate of the casein. Fat in the preparations may be polar lipid molecules bound to the protein. But fat globules that somehow have obtained some plasma protein (mostly casein) in their surface coats, easily may become entrapped in the protein mass, and this makes up most of the fat in most preparations.

### 17.7.1. Types of Preparations

The properties of the preparations depend on those of the proteins included (Chapters 6 and 13 and Section 10.3) and on the method of manufacturing. We will deal with the latter only so far as needed for a qualitative understanding of product properties.

*Casein* usually is isolated from skim milk by adding acid (HCl or lactic acid) to the isoelectric pH. In this way most of the calcium phosphate and the proteose–peptone in the micelles is lost. The acid casein can be washed, pressed to remove water, and then dried. Such a product is rather insoluble. The acid-precipitated casein also can be dissolved in alkali (pH ~ 7) and spray dried. Na, K, and Ca caseinate are produced this way. Solubility and

**Table 17.3. Examples of the Composition of Milk Protein Preparations**

| Product | Method of Preparation[a] | Isolated from | Composition[b] | | | |
|---|---|---|---|---|---|---|
| | | | protein (%) | carbohydrate (%) | "ash" (%) | fat (%) |
| Acid casein | acidification | skim milk | 83–95 | 0.1–1.0 | 2.3–3.0 | ~2 |
| Na caseinate | acid + NaOH | skim milk | 81–88 | 0.1–0.5 | ~4.5 | ~2 |
| Rennet casein | renneting | skim milk | 79–83 | ~0.1 | 7.0–8.0 | ~1 |
| WP[c] concentrate | ultrafiltration | whey | 50–75 | 8.0–40.0 | 1.0–6.0 | 2–10 |
| WP concentrate | electrodialysis[d] | whey | 27–37 | 40.0–60.0 | 1.0–10.0 | ~4 |
| Whey powder[e] | spray drying | whey | 11 | 73.0 | 8.0 | 1 |
| WP complex | metaphosphate | whey | 55 | 13.0 | 13.0 | 5 |
| WP complex | CMC | whey | 50 | 20.0[f] | 8.0 | 1 |
| WP complex | Fe + polyphosphate | whey | 35 | 1.0 | 54.0 | 1 |
| Lactalbumin | heat + acid | whey | 78 | 10.0 | 5.0 | 1 |
| Coprecipitate | heat + acid | skim, buttermilk | 85 | 1.0 | 8.0 | 2 |

[a]Always includes drying
[b]Water content 3–12%
[c]WP = whey protein
[d]And lactose crystallization
[e]Shown for comparison
[f]Includes CMC (carboxymethyl cellulose)

flavor can be good, if the product is made from fresh (undried) and well-washed casein, and if the pH during isolation, washing, and drying is never too high (> 7). Na caseinate is the most common product; K caseinate sometimes is preferred for nutritional purposes; Ca caseinate has somewhat different characteristics (see below).

Casein, or rather paracasein, also can be isolated from skim milk by renneting, syneresis, washing the curd, and drying. This so-called rennet casein is insoluble under most conditions and has a high content of calcium phosphate.

*Whey proteins* can be concentrated in various ways. A much-applied method is ultrafiltration of whey; the mode of operation called diafiltration (Section 17.6) gives a fairly pure protein. Other ways are removal of lactose from whey by crystallization and of salts by electrodialysis or ion exchange. Whey proteins also can be adsorbed onto some ion exchange resins at neutral pH and desorbed at low pH; the protein thus isolated mainly comprises $\beta$-lactoglobulin and $\alpha$-lactalbumin. The preparations obtained after spray drying are called whey protein concentrates; they are highly soluble.

Whey proteins can be precipitated at low pH by carboxymethyl cellulose (CMC) or with hexametaphosphates. The products so obtained are called complexes; they contain the precipitating agent and have low solubility at low pH. (See Figure 17.9.) The proteins also can be precipitated with ferric ions at low pH or with ferric ions and polyphosphates at neutral pH; such products have a very high ash content (Table 17.3) and poor solubility.

The solubility of the proteins closely depends on the extent of heat denaturation, though in relation to the $Ca^{2+}$ ion concentration and the pH during denaturation. At high pH and low $[Ca^{2+}]$ denaturation does not cause appreciable loss of solubility. (See Figure 17.9.) Electrodialysis seems to cause extensive insolubilization of the $\alpha$-lactalbumin. About half of a whey protein preparation consists of $\beta$-lactoglobulin, and its properties dominate those of the mixture (for instance, the conditions leading to and the effects of heat denaturation). In ultrafiltration retentates, about 5% of the N is nonprotein

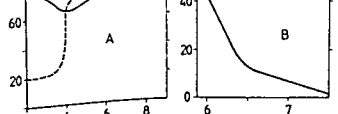



**Figure 17.9.** "Solubility" of whey protein concentrates and a whey protein–CMC complex (— — —); 1% dispersions in water. (A) Percentage soluble as a function of pH; (B) percentage insoluble after heating (10 min at 80°C) at different pH. Approximate examples after various sources.

N, most of this N is probably in the caseino-macropeptide cleaved from κ-casein by rennet action The whey protein complexes contain less NPN, say, 1% If a whey protein solution is desalted effectively by electrodialysis, the ionic strength may become so low as to cause a decreased solubility of β-lactoglobulin and the immunoglobulins The latter may precipitate partially, thereby effectively removing lipids (about 90%) and particulate material (including some denatured protein) from the solution In this way a product with fairly superior properties (solubility, foaming characteristics) can be made

It has long been a practice in some countries to heat sour cheese whey in order to recover the insolubilized whey protein as a precipitate or a scum, it is used to obtain cheeselike products A similar process is applied to obtain a protein preparation called *lactalbumin* not to be confused with the whey protein α lactalbumin, as the preparation also contains β-lactoglobulin, serum albumin, and immunoglobulins, all denatured Proteose–peptone and whey proteoses are not included Precipitation is achieved by adding HCl and/or $CaCl_2$ to the whey and heating it (e g , 30 min at 90°C) A considerable quantity of lactose is included, partly in modified form because of Maillard reactions The protein is insoluble

The same treatment (adding acid and/or $CaCl_2$ and heating) applied to skim milk, sweet cream buttermilk, or mixtures gives a *coprecipitate* that includes the casein and the heat denaturable whey proteins Like lactalbumin it is largely insoluble, but if carefully made it contains far less lactose and Maillard products The swelling characteristics closely depend on the amount of $CaCl_2$ used for the precipitation Coprecipitate is high primarily in biscuits and other dry foods

### 17.7.2. Functional Properties

Protein preparations are used in food manufacture to obtain products with certain desirable properties, which vary widely among foods The suitability of proteins to bring about the desired properties also varies widely The suitability of a certain protein preparation will depend on the composition of the food and on its way of manufacturing and storage Nevertheless, authors have tried to define characteristic features of the protein that can be determined and that have some general validity Most authors have their own classification of these functional properties, ours is below

**Nutritional Quality.** The amino acid pattern is the hallmark of the nutritional quality of a protein and it is clear (Section 19 5) that the quality of casein is good, that of whey proteins better, and that of whole milk protein better still Whey protein concentrates are widely used in baby foods to give them a protein composition more like that of human milk Loss of available lysine may vary widely, according to manufacturing and storage conditions

and product composition (lactose content, pH, $a_w$). In a well-made and well-stored product the loss is generally slight. Caseinates that undergo severe heating during manufacture, particularly at high pH, may contain appreciable quantities of lysinoalanine (see Section 10.3), a substance toxic for rats though not for humans. Contamination with heavy metals may occur, particularly if the pH during manufacture is low, but can easily be avoided by the use of proper equipment.

**Flavor.** Pure proteins have no flavor. Other constituents and protein deterioration are thus responsible for any flavor the preparations may have. Deterioration is like that of other dry milk products (Section 17.2.2), according to composition. Water activity is not always low enough to prevent microbial and enzymic proteolysis; this is clearly avoidable. Maillard reactions (Section 10.4) may occur and are probably responsible for the gluey or stale flavors encountered in some caseinates; again, they can be largely avoided. Dissolved whey protein concentrates are prone to development of sunlight flavor. Lipid autoxidation may occur in products contaminated with Cu.

**Solubility.** Good solubility generally is desired for proteins used in liquid foods. It is, of course, a function of conditions like pH and interaction with other components in the food. To estimate solubility, we often make dispersions of the protein preparation in water and determine which fraction of the protein does not sediment in a centrifuge test. This fraction is then called the solubility, though the definition differs from the common one applied in chemistry. Some examples for whey protein preparations are in Figure 17.9. The solubility of caseinates strongly depends on pH, salt content, and type of cation. Examples are in Figure 17.10. Caseinate can be salted out at fairly low salt concentrations, where undenatured whey proteins stay in solution. Denatured whey proteins are much more sensitive to, for instance, $CaCl_2$.

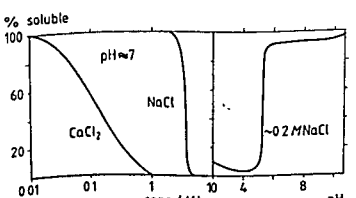



**Figure 17.10.** "Solubility" of Na caseinate (3% dispersions) as a function of salt concentration and pH. Approximate results after various sources.

In some uses (dry foods, baked products) the proteins need not be soluble, coprecipitate and lactalbumin are especially suitable

**Gelling Properties.** In other uses, a protein solution or dispersion should become a gel or a solid mass in the product Whey proteins in solution become insoluble by heating, particularly near their isoelectric pH and at high [$Ca^{2+}$] above the isoelectric pH The protein concentration has to be rather high for a gel to be formed Because whey proteins contain free thiol groups, intermolecular –S–S– cross links can be formed if the pH is sufficiently high (say > 8) This causes gelation in a similar way as that which occurs on heating of eggwhite Caseinate solutions can be made to gel by acidification, renneting, or with $CaCl_2$ Some examples of rheological properties of skim milk gels are given in Figure 16 3, and they essentially apply to caseinate gels as well Ca caseinate is made insoluble by adding orthophosphate, and this can be applied in texturizing of fiber spinning

**Swelling.** Swelling, or water holding, is not the same as water binding or hydration (See Section 17 1 2 ) In many foods, the quantity of water that a given quantity of protein can imbibe is of importance Again, it very much depends on environmental conditions Casein tends to expand at high pH, low $Ca^{2+}$ activity, and low temperature Section 13 1 gives particulars about the voluminosity of micellar casein Serum proteins show little swelling, they are compact molecules On denaturation they imbibe more water, say 2 g per gram of protein The swelling of a coprecipitate depends much on its Ca content, high Ca going along with little swelling

**Viscosity of solutions of the proteins.** This is primarily a function of their concentration, the state of swelling of the protein molecules, and any aggregation General aspects are discussed in Sections 16 1 and 16 2 Figure 16 2 gives some idea about the viscosity of whey protein solutions, because whey proteins exhibit little swelling viscosity is comparatively low Solutions of CMC–whey protein complex are much more viscous Viscosity of caseinate solutions is shown in Figure 17 11 the effects of temperature and pH are striking The strong effect of concentration and the shear thinning are typical for macromolecules that have a fairly extended conformation The viscosity greatly depends on the presence and concentration of cations, Ca caseinate gives less viscous solutions than Na caseinate The increase in viscosity caused by adding NaCl remains unexplained

**Emulsifier properties.** Some aspects should be distinguished (see also Section 14 4)

1. Different proteins give layers around the oil globules of different thickness or protein load (mass of protein per unit oil surface area) This primarily determines the amount of protein needed to produce

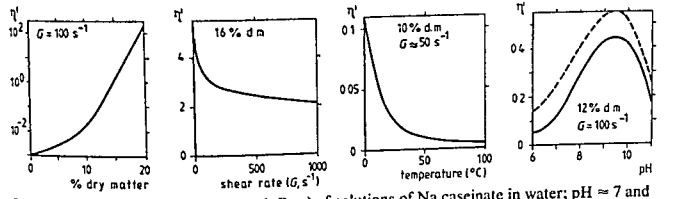



**Figure 17.11** Apparent viscosity ($\eta'$, Pa·s) of solutions of Na caseinate in water; pH ≈ 7 and room temperature, unless stated otherwise. $G$ is shear rate. The broken line pertains to solutions in 0.2 $M$ NaCl. Approximate examples after various sources.

an emulsion. Most globular proteins and well-dissolved caseinates give a protein load of some 2.5 mg·m$^{-2}$, while aggregated proteins (Ca caseinate; casein micelles as in skim milk) may give far higher values, and a poorly soluble protein cannot be used for emulsification. For Na caseinate, the conformation is important; for instance, a higher ionic strength gives a more compact conformation, hence a higher protein load. There will be comparable effects of pH. The protein load also depends on the amount of protein available per unit oil surface area created. Figure 17.12A gives some examples.

2. The size of the globules obtained may depend on the type of protein and on its concentration, or rather on the ratio of protein to oil; examples are in Figure 17.12B. Because specific surface area is inversely proportional to average globule diameter ($d_{vs}$), the relation between protein concentration and protein load, hence amount of protein needed to obtain globules of a certain size and protein load, is usually intricate. If the protein is relatively abundant, the globule

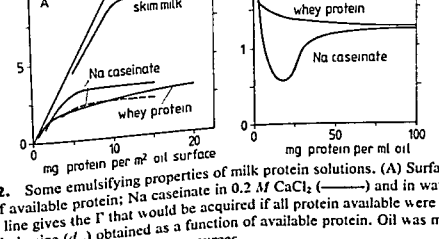



**Figure 17.12.** Some emulsifying properties of milk protein solutions. (A) Surface load ($\Gamma$) as a function of available protein; Na caseinate in 0.2 $M$ $CaCl_2$ (———) and in water (— — —). The straight line gives the $\Gamma$ that would be acquired if all protein available were adsorbed. (B) Average globule size ($d_{vs}$) obtained as a function of available protein. Oil was mostly paraffin oil. Approximate examples after various sources.

size obtained depends primarily on emulsifying conditions (stirring rate, homogenizing pressure etc )

3 The stability of the globules toward coalescence closely depends on type of protein and protein load This is the reason for the (variable) effect of protein to oil ratio (if low) on globule size obtained, if the globules formed are very unstable they already coalesce during or shortly after emulsification It should be taken into account that emulsions with smaller globules and with a lower volume fraction of oil are more stable Na caseinate gives extremely stable emulsions, presumably the exposure of both hydrophobic and hydrophilic regions of the protein molecules causes a strong adsorption of them onto the oil–water interface (Section 12 1 2) and considerable extension into the aqueous phase with ensuing steric repulsion (Section 12 2 4) Emulsions made with whey proteins are less stable, particularly if protein load is low and temperature high, if the pH is between 4 and 5 (i e , near the isoelectric pH), the emulsion stability is so poor that no emulsion can be made Most heat denatured whey proteins are poor stabilizers of emulsions though denaturation at high pH is not so detrimental

4. The emulsion globules may aggregate This can happen afterward when changed conditions cause the protein to become insoluble For instance globules coated with caseinate will flocculate on adding rennet bringing to low pH, or adding much CaCl Aggregation during emulsification also may occur this concerns homogenization clusters (See Section 14 4 3 ) They will be formed if the protein to-oil ratio is low and the protein is (partially) aggregated

It may be concluded that Na caseinate has excellent properties as an emulsifier If somewhat less stable emulsions are wanted whey proteins can be used or small molecule surfactants can be added in small quantities as they diminish stability toward coalescence

**Foaming** This is discussed in Chapter 15 The tendency of a protein solution to foam may parallel its usefulness as an emulsifier For instance, the solubility should be good in both cases Important foaming properties are the overrun that can be obtained (e g 2000% with some proteins) and the foam stability against collapse and drainage To minimize drainage a high viscosity of the protein solution is desirable The stability against collapse is less well understood but the presence of even small quantities of lipids generally is detrimental

Na and K caseinate may give copious and stable foams if no lipid is present Ca caseinate is not suitable Partly hydrolyzed casein (usually hydrolyzed by mild heating under alkaline conditions) gives even more stable foams again in the absence of lipids

Undenatured whey proteins give fairly copious and stable foams though

lipids and aggregates present are detrimental. Consequently, a defatted and clarified whey protein concentrate is much superior. The foaming properties do not depend much on pH. Heat-denatured whey proteins give less foam, particularly if denatured at low pH, but foam stability is better. Undenatured whey proteins also may give foams that are fairly resistant to heating (baking).

## 17.8. RECONSTITUTION AND RECOMBINATION

If a concentrated milk product is diluted with or dissolved in water to its original composition, it is said to be reconstituted. The term *reconstituted milk* usually implies milk obtained by dissolving milk powder. It may take considerable time, say, 24 h, before some reversible changes occurring during evaporation and drying are reversed; this mainly concerns the distribution of salts among casein micelles and serum, and the size and structure of the micelles. The irreversible changes (off-flavors, loss of nutrients) that have occurred during manufacture or storage of the concentrated milk or powder are retained, of course, in the reconstituted product. Loss of solubility of the protein and coalescence or partial coalescence of the fat globules are particularly cumbersome, because the reconstituted liquid will be inhomogeneous. (Therefore, whole milk powder should be made from well-homogenized milk.) The quality of the water used also has to be considered, both from a microbial and a chemical point of view (off-flavors, hardness). A milk of high fat content, such as buffalo's milk, can be mixed with reconstituted skim milk (from cow's milk) to make a beverage of desirable composition; this is called *toned milk*. Some reconstituted skim milk is consumed as beverage milk because it is cheaper; it is deficient in vitamins A and D unless these have been added to the powder.

An important property of the milk or skim milk powder for its use in reconstitution is the ease of dissolving it in water. If the water is hot and a high-speed stirrer is used, dissolution causes no great problem. But dissolving the powder in cold water under household conditions may be far from easy. "Instant" powder, which rapidly dissolves in cold water with gentle stirring, can be manufactured. The instant property is not related to the solubility but to rapid penetration of water into a mass of powder. The powder particles then disperse in the water where they can dissolve later. Instant properties depend on a number of phenomena occurring when a mass of powder is brought into water:

1. The powder should be wettable by water. This property is determined by the contact angle ($\theta$, as measured in the aqueous phase) of the three-phase system of dry milk, water, and air. The contact angle is discussed in Section 12.1.4. (See especially Figure 12.2.) If $\theta$ is less than 90°, the particles are wettable. For dried skim milk $\theta$ is about 20°, for dried whole milk about 50°. This would imply that water is always sucked into the pores between the powder particles by cap-

illary action In skim milk powder this is always the case. But the wetting of a mass of powder differs from the wetting of single particles, for reasons not fully understood Usually, the effective $\theta$ of the powder is much higher In whole milk powder effective $\theta$ may be greater than 90° (particularly if part of the fat is solid) so that water does not penetrate the powder mass or does so only locally A common remedy is to cover the powder particles with a thin layer of lecithin, thereby diminishing effective $\theta$

2. The rate of penetration of water into the powder depends on the width of the pores between the particles The smaller the particles, the narrower the pores and the slower the penetration A wide range of particle sizes also may cause a small pore size, as the smaller particles fill the voids between the larger ones In a normal spray-dried (skim) milk powder the pores are too narrow to permit rapid penetration
3. Problem 2 above is aggravated because the capillary action of the penetrating water pulls the wetted particles closer to each other, thereby narrowing the pores This capillary contraction can cause a reduction in volume of the powder of as much as 30–50% The swelling of protein by water uptake also may cause a narrowing of pores, especially in dried protein preparations
4. The penetration of water is slowed down further because part of the dried milk, especially the lactose, dissolves, producing a very viscous solution The lactose can dissolve very quickly because it is present in an amorphous state, we may say that it becomes diluted rather than dissolved The combined action of 2, 3, and 4 soon causes the penetrating water front to stop moving In this way powder lumps are formed, dry inside and with an outer layer of highly concentrated milk
5. Other properties of the powder may be of some influence, such as whether adjoining particles will fall apart easily when completely wetted, and whether a particle's density allows it to sink (depending on its vacuole volume) But these aspects usually cause no problems

Besides altering the wettability (contact angle) in the case of whole milk powder, instant properties can be imparted by altering the particle size This is achieved by agglomeration, powder particles that are not (yet) fully dry will stick together when they meet each other, and this is put to use by letting the particles collide and thus aggregate The agglomerates so formed are often up to 1 mm in diameter, and now the pores between them are wide enough The agglomerates should be sufficiently strong to withstand mechanical damage during handling of the powder The agglomerates are dispersed easily in water and afterward dissolve slowly

Reconstitution of whole milk powder has some disadvantages Dissolving the powder in cold water is cumbersome Flavor deterioration during storage

of the powder may be considerable; this concerns particularly fat autoxidation, and to minimize it expensive packing methods and an intense preheating are needed. The latter causes a distinctive cooked flavor. Skim milk powder can be kept for a long time without deteriorating (if its water content is sufficiently low), and the skim milk need not be high heated before drying. Anhydrous milk fat also has good keeping quality if well prepared. Consequently, it has become practice to use these ingredients and water to make *recombined milk*. This amounts to reconstituting skim milk and emulsifying milk fat into it, at a temperature (> 40°C) at which the fat is liquid. Emulsifying can be achieved by means of a high-speed stirrer, though a homogenizer often is used; there is no need to add emulsifiers. The composition of recombined milk differs from that of natural milk in that the components of the fat globule membrane are lacking. This implies a good stability to fat autoxidation but also a difference in flavor (the recombined milk tastes somewhat like skim milk). The composition and flavor can be made more like that of natural milk by replacing part (usually 5–10%) of the skim milk powder with sweet-cream buttermilk powder, which is rich in fat globule membrane components (notably phospholipids). The fat globules of recombined milk differ considerably from the natural ones, as is discussed in Section 14.4. (See especially Table 14.3.) This has important consequences for the properties of recombined milk with respect to heat and emulsion stability and to the rheological properties after renneting and souring the milk. The fat globules can be made more like natural ones by emulsifying them into a fairly small volume of reconstituted sweet-cream buttermilk and then adding reconstituted skim milk.

The properties of recombined milk depend on processing conditions (such as homogenization pressure) and on the properties of the raw material. An important feature of the skim milk powder used is the heat treatment it has received, not so much during drying (where the heat treatment it has received is usually negligible) as before. One aspect is absence of cooked flavor; others are rennetability and cold agglutinability. These properties go along with little or no heat denaturation of the whey proteins. For some uses we may want an intensely heat-treated powder, for instance, to inactivate deteriorative enzymes, to kill microorganisms (most of them survive drying and subsequent storage), or to impart heat stability. A common classification is based on denaturation of whey proteins, as determined by the Harland–Ashworth test. This so-called WPN index roughly measures the amount of undenatured whey proteins. If it is $\geq 6$ mg $N \cdot g^{-1}$ of skim milk powder, the powder is called "low heat," if $\leq 1.5$ mg $N \cdot g^{-1}$ it is "high heat," and in between it is "medium heat." The test is not very precise. The WPN index for fresh milk ranges from some 6.5–10 (recalculated for skim milk powder). Consequently, in a "low heat" powder a substantial portion (up to 40%) of the denaturable whey protein may have been denatured, in a "high heat" powder up to 15% not. Nevertheless, the index gives some idea about the heat treatment.

An important property of the milk fat as a raw material for recombined milk is absence of off flavors Another feature is melting range (See Section 5 7 1 ) The range can be altered by fractional crystallization, and in this way functional properties of some products can be improved (for instance, whippability of recombined cream) We may even use a fat other than milk fat, to reduce the price or for nutritional purposes The resulting product is called *filled milk*

Other recombined products can be and are being made, such as evaporated milk, sweetened condensed milk, and ice cream mix The skim milk powder used often must have been made in a specific way, especially with respect to heat treatment The principle of recombination offers possibilities for making products in a wide compositional range Not only can the fat be modified or altered, but the same holds true for the solids-not fat We may use whey powder, whey protein concentrate, sodium caseinate, and many others to obtain specific nutritional and functional product properties (See also Section 17 7 )

## Suggested Literature

General aspects of most processes discussed in this chapter are treated in

A Spicer ed *Advances in Preconcentration and Dehydration of Foods* (London Applied Sci Publ 1974)

Several aspects are also discussed in

M Karel D B Lund and O R Fennema *Physical Principles of Food Preservation* (New York Dekker 1975 (second edition in preparation)

Water relations of foods and their effects on food properties and stability are treated in two symposium reports

R B Duckworth ed *Water Relations of Foods* (London Academic Press 1975)

L B Rockland and G F Stewart eds *Water Activity Influences on Food Quality* (New York Academic Press 1981)

General aspects of freezing of foods are given by

O R Fennema W D Powrie and E H Marth *Low Temperature Preservation of Foods and Living Matter* (New York Dekker 1973)

Freezing of milk and milk products is discussed in

P G Keeney and M F Kroger Chapter 14 in B H Webb A H Johnson and J A Alford eds *Fundamentals of Dairy Chemistry* 2nd ed (Westport AVI 1974) p 873

Chapters 9 and 11 of that book also give some aspects relevant to the present chapter for instance on evaporated milk

Properties of dry milk products are reviewed by

S T Coulter and R Jenness in vol 2 of W B van Arsdel et al eds *Food Dehydration* 2nd ed (Westport AVI 1973) pp 290–346

A series of articles on physical properties of milk powders is by

T J Buma *Neth Milk Dairy J* 25 (1971) 33 42 53 75 88 107 123 151 and 159

Two reviews on reverse osmosis and ultrafiltration in dairying are by

F. A. Glover, P. J. Skudder, P. H. Stothart, and E. W. Evans, *J. Dairy Res.* 45 (1978): 291.

R. A. M. Delaney and J. K. Donnelly, Chapter 20 in S. Souriragan, ed., *Reverse Osmosis and Synthetic Membranes* (Canada: Nat. Res. Council, 1977), pp. 417–41.

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A general survey of functional properties of proteins in foods is by

J. E. Kinsella, *Crit. Rev. Food Sci. Nutr.* 7 (1976): 219.

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A. van Kreveld, *Neth. Milk Dairy J.* 28 (1974): 23.

# 18

# Flavors and Off-Flavors

Chapter contributed by H T Badings

The most important factor that governs appreciation or dislike of foods is flavor, the complex sensation comprised of aroma, taste, and aspects of texture The dairy industry is well aware of the importance of flavor as an essential factor in food selection and acceptance, much effort has been put into the development of methods for quality control of the flavor of starting materials and end products and into detection of flavor changes resulting from processing, packaging, and storage Also, studies have been made to identify flavor compounds in milk and dairy products, to determine their significance, and to elucidate factors and mechanisms underlying their development

In the past, flavor quality control was effected mostly by sensory evaluation, supported by microbiological analysis and the determination of chemicoanalytical indices of such deteriorative changes as oxidation, lipolysis, and heating These methods certainly have their merits, but it is clear that identification of compounds responsible for particular flavors and aromas may deepen our knowledge in this field offer the opportunity to improve the sensory quality of foods, and help explain the mechanisms of flavor deterioration

Before reviewing the flavor compounds found in milk and dairy products, some basic aspects of flavor perception, flavor research, and the principle of favor balance will be considered briefly

## 18.1. GENERAL ASPECTS

### 18.1.1. Flavor Perception

Flavor is the complex sensation that includes taste, aroma, and texture These three elements are detected by the gustatory, olfactory, and tactual

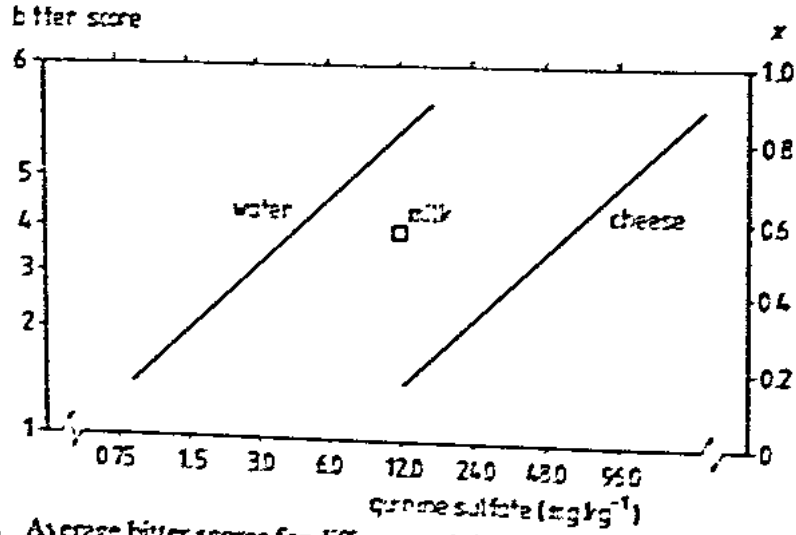



**Figure 18.2.** Average bitter scores for different quinine sulfate concentrations in water, milk, and cheese. Quinine sulfate concentrations are given on a logarithmic scale. To obtain a linear relation, the bitterness score (Sc = 1–6) has been transformed according to $x = \arcsin \sqrt{(Sc-1)/5}$/arcsin 1. After F. M. W. Visser, *Neth. Milk Dairy J.* 31 (1977):200.

strong for olfactory stimuli. Reinforcement means that the sensory response to a flavor compound can be intensified by the presence of other constituents. The other component need not impart flavor itself; a good example is $CO_2$. Negative reinforcement, that is to say, masking or extinguishing effects in mixtures of flavor compounds, also has been observed.

Apart from the variance among individuals in quantitative flavor sensitivity, there is also a significant difference in their response in terms of appreciation or depreciation for certain flavor stimuli. The acceptability of a certain food flavor may vary with individual differences in the sensitivity to that flavor (i.e., threshold) and in the intensity of its perception. Other factors that are very important in an individual's attitude toward a particular flavor or food include (1) food or eating experience (including conditioning or other learning effects by education, socioeconomic status, etc.), (2) psychological motivation (a matter of experience, personality, eating environment, etc.), and (3) physiological condition (hunger, illness, etc.). The food industry has to take these aspects into consideration. Consumer acceptance tests are used to determine the extent of appreciation of a certain product for a certain market.

### 18.1.2. Identification of Flavor Compounds

The mixture of odorous compounds present in food products is usually very complex. It may consist of hundreds of individual components, mostly in very low concentrations ($\mu g \cdot kg^{-1}$ to sub $pg \cdot kg^{-1}$). Yet many of them together contribute to the sensory properties of foods. The low concentration and instability of many aroma compounds make it difficult to obtain a flavor concentrate from the bulk of the food product, which is representative of the original flavor and free from contaminants and artifacts. Special tech-

**Table 18.1. Flavor Threshold Values of Compounds Contributing to (Off)-Flavors in Milk and Milk Products**

| Compound | Flavor Threshold (mg/kg) | | |
|---|---|---|---|
| | In Water | In Oil | In Milk |
| *n*-Alkanoic Acids | | | |
| 2 | 50 | | |
| 3 | 3 | | |
| 4 | 7 | 0 5 | 25 |
| 6 | 5 | 2.5 | 14 |
| 8 | 6 | 350 | 10 |
| 10 | 3 5 | 200 | 13 |
| 12 | | 700 | 9 |
| Aldehydes (*n*-Alkanals) | | | |
| 2 | 1 00 | | 1.50 |
| 3 | 0 06 | 1.5 | 0 50 |
| 4 | 0 06 | 0 03 | 0 20 |
| 5 | 0 01 | 0 15 | 0.15 |
| 6 | 0 02 | 0 1 | 0 07 |
| 7 | 0 01 | 0 06 | 0 10 |
| 8 | 0 001 | 0 1 | 0.10 |
| 9 | 0 005 | 0 3 | 0.20 |
| 10 | 0 005 | 0 7 | 0.30 |
| 6Δ[2t] | | 1 0 | 0 10 |
| 8Δ[2t] | | 0 5 | |
| 9Δ[2t] | | 0 05 | 0 004 |
| 6Δ[2c] | | 0 1 | |
| 7Δ[2c] | | 0 001 | |
| 7Δ[2t,4t] | | 0 1 | |
| 10Δ[2t,4t] | | 0 1 | 0 001 |
| 10Δ[2t,4c] | | 0 02 | |
| 9Δ[2t,4c] | | | 0 002 |
| Alcohols (*n*-Alkanols) | | | |
| 3 | 7 0 | | |
| 4 | 6 0 | 50 | 0 5 |
| 5 | 5 0 | 50 | 0 4 |
| 6 | 0 5 | 5 | 0.5 |
| 7 | 2 4 | 5 | 0 6 |
| Lactones | | | |
| γ6 | 0 1 | 4 | |
| δ6 | 0 6 | 3 | |

Table 18.1. (*Continued*)

| Compound | Flavor Threshold (mg/kg) | | |
|---|---|---|---|
| | In Water | In Oil | In Milk |
| γ10 | 0.1 | 2 | 1.5 |
| δ10 | 0.2 | 2 | 2.0 |
| δ12 | 0.1 | 95 | |
| **2-Alkanones (Methyl Ketones)** | | | |
| 4 | 3.0 | 20.0 | 80.0 |
| 5 | 0.3 | | 9.0 |
| 6 | 0.1 | 5.0 | 0.4 |
| 7 | 0.2 | 0.7 | 0.7 |
| 8 | 0.1 | 0.6 | 0.5 |
| 9 | 0.1 | | 4.0 |
| 10 | 0.01 | | 15.0 |
| 11 | 0.03 | | 20.0 |
| **Ethyl Esters** | | | |
| 4 | | 0.5 | 0.03 |
| 6 | 0.01 | 0.7 | 0.08 |
| **Miscellaneous** | | | |
| 1-octen-3-one | | 0.1[a] | 10.0[a] |
| 1-octen-3-ol | | 10.0[a] | |
| diacetyl | 5.0[a] | 50.0[a] | 20.0[a] |
| dimethyl sulfide | 10.0[a] | 40.0[a] | 20.0[a] |
| methanethiol | 0.1[a] | | |
| methional | | | 10.0[a] |
| o-amino-acetophenone | | | 0.5[a] |
| skatole | 50.0[a] | | |
| indole | 100.0[a] | | |
| benzthiazole | 80.0[a] | | |
| 2,4-dithiapentane | 0 3[a] | 3[a] | |
| 5-hydroxymethyl-furfural | 5.0 | | 1.0 |
| phenyl-acetaldehyde | 9.0[a] | | |

*Note.* Average values from various sources.
[a]Values expressed as μg/kg (other values in mg/kg).

niques for the isolation and concentration of flavor compounds have been developed. Most of them are based on low-temperature vacuum distillation, degassing, vapor entrainment or head-space techniques, solvent extraction, freeze concentration, and adsorption.

For the separation of flavor concentrates into the individual components, gas chromatography (GC), particularly with glass or fused silica capillary columns, is the most suitable method. The separated components usually are identified by spectrometric analysis such as mass spectrometry (MS), particularly by directly coupled GC–MS, infrared spectrometry (IR), and nuclear magnetic resonance spectrometry (NMR).

It is doubtful whether the published results always have been obtained by methods that exclude artifact formation sufficiently to make identification of original aroma constituents certain.

### 18.1.3. Correlation of Flavor Analysis with Sensory Characteristics and the Concept of Flavor Balance

Chemicoanalytical methods alone cannot reveal the significance of aroma compounds for the flavor of a product, nor can they indicate elements of like–dislike in food appreciation. It is evident that correlations between the sensory properties of foods and the analytical data on the component flavor constituents should be determined experimentally in order to obtain full profit from flavor identification research. The flavor contribution of identified aroma compounds should be determined qualitatively (flavor note) and quantitatively (flavor intensity). The quantity of a flavor compound compared to its flavor threshold concentration may be indicative of its contribution to the flavor of a product, but this relation should be used with care. The overall impression is not simply the sum of the component parts. The intensity perceived is related to the concentration of the stimulus (which is influenced by binding, compartmentalization, release during eating, etc.) according to the function proposed by Stevens:

$$R = n\log S + \log c \qquad (18.1)$$

in which $R$ is the response (sensation), $S$ is the concentration of the stimulus, and $n$ and $c$ are constants. (It should be noted that, in fact, thermodynamic activity rather than concentration of the stimulus determines the response.) Extinguishing and reinforcing effects also have been observed in mixtures of flavor compounds. Furthermore, flavor contributions of aroma compounds strongly depend on the medium in which the latter are dispersed and on the extent to which they are bound or adsorbed.

One aspect that has become clear from flavor research work is the principle of flavor balance. It is the delicate balance among the many aroma compounds, most of which are present in subthreshold concentrations, that produces the desirable flavor of a particular food product. If this balance is disturbed an off-flavor may occur. A difference between two batches of a product, one of good sensory quality and one with an off-flavor, often may

be a matter of quantitative rather than qualitative difference in flavor compound composition. Examples of this are given in the discussion of the flavor of milk and dairy products and of certain defects. Of course, there are also flavor defects that result from specific contaminations or are caused by specific compounds produced in chemical, enzymatic, or microbial processes.

It is desirable to know more about the influence of the physical structure of products on flavor (e.g., the effect of homogenization of milk on its flavor), about physicochemical interaction between aroma compounds and constituents of dairy products (e.g., the adsorption and binding of flavor compounds to lipids or proteins), and about the extent to which flavors are released when food products are eaten.

## 18.2. THE FLAVOR OF MILK

Fresh milk (raw or low-temperature pasteurized) of good quality has a bland but characteristic flavor. The sensory perception it brings about is determined mainly by the pleasant mouthfeel from the physical constitution of milk, which is an emulsion of fat globules in an aqueous colloidal protein phase, and a slight salty sweet taste that results from milk salts and lactose. Many odorous compounds, mostly in subthreshold concentrations, constitute the aroma of milk.

Since the finding of Patton that dimethyl sulfide is an important aroma constituent of fresh milk, many aroma compounds have been identified. These belong to many different classes, such as carbonyl compounds, alkanols, fatty acids, lactones, esters, sulfur compounds, N compounds, and aliphatic and aromatic hydrocarbons.

Some recent results of research on milk flavor are given in Figure 18.3, which represents the volatile compounds found in LP milk (low-pasteurized milk heated 12.0 s at 73°C in a tubular sterilizer) and in UHT milk (ultra-high-temperature-treated milk processed by indirect heating for 4.6 s at 142°C in a tubular sterilizer). A condensate of volatiles from the milk was prepared and further analyzed by coupled capillary column GC–MS. At least 400 compounds were found. The odors and flavors of the separated fractions were evaluated in order to determine which compounds contribute to the overall aroma of milk. It is clear that the aroma of milk is determined by many volatile compounds mostly present in very low concentrations below their flavor thresholds. The total aroma concentrate is estimated to be 1–100 $mg \cdot kg^{-1}$. Therefore, the quantity of most of the individual constituents will be less than 1 $\mu g \cdot kg^{-1}$. A number of these compounds may be transferred from the feed to the milk while others result from minor conversions of milk constituents by chemical, microbial and enzymatic, reactions. If the quantity of aroma compounds formed exceeds a certain level, the flavor balance may be upset and off-flavors result. These aspects and the production of different types of flavor in milk by heating are discussed in the following section.

## 18.3. OFF-FLAVORS IN MILK AND DAIRY PRODUCTS

In many milk and dairy products the same processes of deterioration can occur, which may lead to similar types of off-flavor. However, the quantitative composition of the product (e.g., low or high fat content), the physical structure (e.g., emulsion or dried product), the bacterial constitution, the process of manufacturing, and storage conditions can affect greatly the type of deterioration that is prevalent, the course of the reaction, and the perceptibility of any compound causing off-flavors.

### 18.3.1. Off-Flavors Caused by Extraneous Components

**Absorbed Flavors.** Fresh milk may have off-flavors from the transfer of certain components from the feed to the milk. There are two pathways by which flavor compounds may enter the milk through the cow. One is by the pulmonary tract to the bloodstream, to the mammary cells, and into the milk. The other is through the digestive tract to the blood and thence into the milk. It should be noted that odors in a stable (from the feed, from contaminated air, or from substances given off by the cow in the breath, sweat, urine, and feces) may accumulate readily in the milk by the inhalation route and can lead to off-flavors. Also, residual disinfectants may impart off-flavors to milk. The direct transfer of odors by exposing milk to the air in the stable seems to be less significant.

Removal of absorbed flavors, particularly those caused by low-boiling or hydrophilic compounds, is possible by heat and vacuum treatment, but some off-flavors are very persistent.

**Feed Off-Flavors and Weed Taints.** These are caused by increased quantities of benzyl mercaptan, methyl mercaptan, dimethyl sulfide, dimethyl disulfide, indole, skatole, and trimethylamine.

**Cowy Flavor.** Cowy flavor sometimes occurs in raw milk, especially in the winter period. It can be attributed in part to the transfer of taints from the air in the stable or from the feed. However, it seems to be related particularly to ketosis or acetonemia, a disease in cattle involving the endogenous energy metabolism and leading to increased concentrations of acetone in milk.

### 18.3.2. Off-Flavors Caused by Chemical Reactions

**Oxidation Flavor Defects.** An important source of flavor defects in milk and dairy products is oxidation of the lipid fraction. These defects are caused chiefly by increased concentrations of carbonyl compounds resulting from

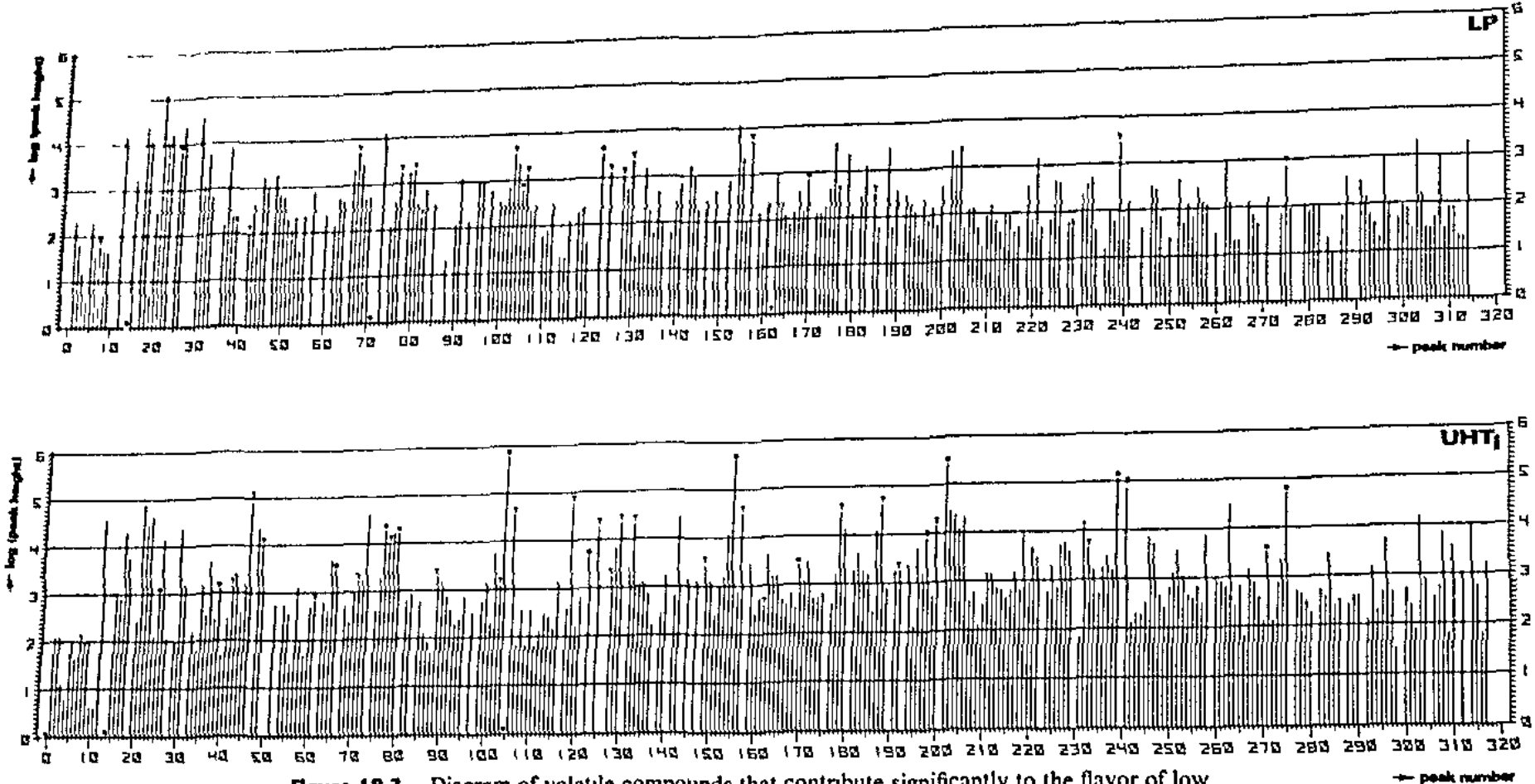



**Figure 18.3.** Diagram of volatile compounds that contribute significantly to the flavor of low pasteurized and UHT-heated milk. After H. Badings and R. Neeter, *Neth. Milk Dairy J.* 34 (1980)·9.

**Identity of Peaks that Contribute to Flavor**

| Peak Number | Compound | LP | UHT |
|---|---|---|---|
| 0 | Hydrogen sulfide | 0 | 2 |
| 7 | Acetaldehyde | 1 | 0 |
| | Methane thiol | 0 | 1 |
| 14 | Dimethyl sulfide | 3 | 3 |
| 25 | Diacetyl | 2 | 3 |
| 39 | 3-methyl butanal | 1 | 2 |
| 42 | 2-methyl butanal | 1 | 1 |
| 46 | Pentanone-2 | 0 | 1 |
| 49 | Pentanal | 0 | 1 |
| | *i*-butyl mercaptan | 0 | 1 |
| 61 | Methyl isothiocyanate | 0 | 1 |
| 66 | Dimethyl disulfide | 0 | 2 |
| 67 | 4-pentene nitril | 1 | 0 |
| 71 | 2-methyl butanol-1 | 2 | 1 |
| 77 | Hexanone-2 | 1 | 3 |
| 78 | Ethyl isothiocyanate | 0 | 1 |
| 80 | Hexanal | 1 | 2 |
| 82 | Ethyl butyrate | 1 | 0 |
| 89 | Furfural | 0 | 1 |
| 103 | 2,4-dithiapentane | 1 | 1 |
| 104 | Heptanone-2 | 0 | 4 |
| 105 | 4-*cis*-heptenal | 2 | 3 |
| 106 | Heptanal | 1 | 1 |
| 119 | Benzaldehyde | 0 | 1 |
| 123 | 2,3,4-trithiapentane | 0 | 2 |
| | 3-butenyl-isothiocyanate | 2 | 0 |
| 125 | benzonitrile | 1 | 1 |
| 128 | 1-octene-3-one | 1 | 1 |
| 130 | octanone-2 | 0 | 1 |
| | 1-octene-3-ol | 1 | 1 |
| 133 | octanal | 0 | 1 |
| 149 | acetophenone | 0 | 1 |
| 155 | nonanone-2 | 0 | 4 |
| 157 | nonanal | 1 | 1 |
| 162 | p-cresol | 1 | 1 |
| 170 | 2-*trans*-nonenal | 2 | 2 |
| 179 | naphthalene | 0 | 1 |
| 185 | 2*t*, 4*t* nonadienal | 1 | 0 |
| 188 | benzthiazole | 0 | 1 |

**Identity of Peaks that Contribute to Flavor (*Continued*)**

| Peak Number | Compound | LP | UHT |
|---|---|---|---|
| 192 | γ-octalactone | 0 | 1 |
| 198 | δ-octalactone | 0 | 1 |
| 200 | decanol-1 | 0 | 1 |
| 202 | undecanone-2 | 0 | 2 |
| 233 | γ-decalactone | 0 | 1 |
| 239 | δ-decalactone | 1 | 2 |
| 241 | tridecanone-2 | 0 | 1 |
| 271 | γ-dodecalactone | 0 | 2 |
| 275 | δ-dodecalactone | 1 | 3 |

*Note 1:* In diagram: • = (very) strong flavor contribution; o = moderate contribution; T = slight contribution. Peaks without markings are (probably) not important for flavor or give only a slight contribution. The intensity of peaks 13 and 14 is not given, because solvent peaks appear here in the chromatogram.

*Note 2:* In list of compounds: peak number is given followed by the name of the compound. This is followed by the notation for LP milk and for UHT milk. Notations used are: 0 = no contribution, or uncertain, 1 = slight contribution to flavor, 2 = moderate, 3 = strong, and 4 = very strong.

the oxidative breakdown of unsaturated fatty acids. As the mechanism of (aut)oxidation is discussed in more detail in Section 5.6, only the pathway of formation of aroma compounds by these reactions will be given, with linoleic acid as an example. (See Figure 18.4.) In the autoxidation of linoleic acid the C-11 methylene group is the preferential point of attack. The intermediately formed free radical (I) is stabilized by resonance, and the three hybrid structures (I, II, and III) all contribute to the formation of hydroperoxides. The latter may decompose to aldehydes: hexanal, 2-*cis*-octenal (which may readily transform into its *trans* isomer) and 2-*trans*,4-*cis*-decadienal (partly changing into the *trans*, *trans* isomer).

From the different unsaturated fatty acids in milk fat many carbonyl compounds can be formed along this way. The latter may contribute to typical oxidation flavors. Examples are given in Table 18.2. It should be noted that a very limited oxidation of the lipids in milk and dairy products can contribute to desirable flavors. Here again, the principle of the flavor component balance is valid; very low concentrations of 4-*cis*-heptenal (1 $\mu g \cdot kg^{-1}$) may contribute to a full creamy flavor. The same holds for compounds like 1-octen-3-one and hexanal. If, however, the concentration of these compounds passes a certain level, oxidation flavor defects occur (cardboard, metallic, green).

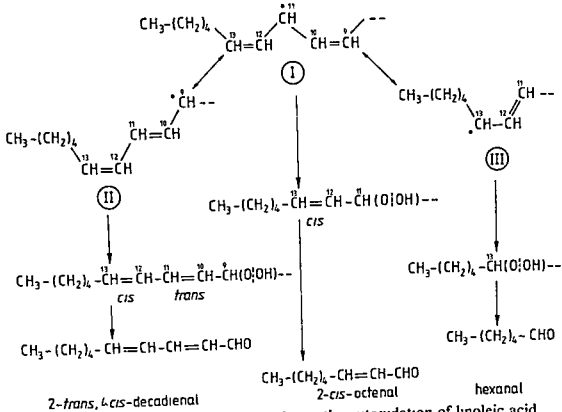



**Figure 18.4.** Formation of aldehydes in the autoxidation of linoleic acid.

**Table 18.2. Compounds Contributing to Typical Oxidation Flavors**

| Compounds | Flavors | Example of Fatty Acid Precursor[a] |
|---|---|---|
| Alkanals $C_{6-11}$ | green, tallowy | most UFA |
| 2-Alkenals $C_{6-10}$ | green, fatty | most UFA |
| 2,4-Alkadienals $C_{7-10}$ | oily, deep-fried | Lo, Le |
| 3-*cis*-Hexenal | green | Le |
| 4-*cis*-Heptenal | creamy, putty | $C_{18:2}\ \Delta^{11\ 15c}$ |
| 2,6- and 3,6-Nonadienal | cucumber | Le |
| 2,4,7-Decatrienal | fishy, sliced beans | Le |
| 1-Octen-3-one | metallic | Lo |
| 1,5-*cis*-Octadien-3-one | metallic | $C_{20:5}\ \Delta 5,8,11,14,17$ |
| 1-Octen-3-ol | mushroom | Lo |

*Note:* For the sake of conciseness the tables in this chapter contain abbreviations. Some examples are aldehydes: $C_4$ = butanal, $3MeC_4$ = 3-methylbutanal; esters: $C_4$-methyl = methylbutyrate; alkanols: $C_{1-3}$ = isopropanol; alkenals: $C_3\Delta^2$ = 2-pentenal, $C_3\Delta^{4c}$ = 4-*cis*-heptenal; lactones: $\delta C_{10}$ = δ-decalactone.

[a]Abbreviations used: UFA = unsaturated fatty acids; Lo = linoleic acid; Le = linolenic acid; $C_{18:2}\ \Delta\ 11,15c$ = $C_{18}$ fatty acid with double bonds at 11 and 15 *cis*.

The changes in flavor of dairy products as a result of oxidation also depend strongly on physical constitution and chemical composition. In butter, autoxidation proceeds particularly in the phospholipids of the fat globule membrane material. The oxidation products migrate to the milk fat. This may cause metallic off-flavors at the start of oxidative deterioration. If this process continues, flavor deterioration proceeds in the sequence fatty, trainy (fishy), and, finally, tallowy.

In milk powder the lipid fraction as a whole is oxidized, directly resulting in tallowy off-flavors. In buttermilk only small quantities of phospholipids are present in an aqueous phase. Here, the oxidation may go on to form secondary oxidation products of lower molecular weight, such as 1-penten-2-one, which impart a distinct metallic flavor.

**Heat-Induced Flavors.** These flavors are a separate subject. Mostly, they are the consequence of a thermal treatment to which milk and its products must be subjected in order to obtain sufficient keeping quality (i.e., to delay microbial, chemical, or enzymatic deterioration).

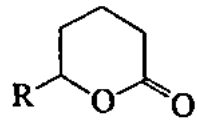
Different types of heating flavor can occur. Low-pasteurized milk (e.g., 73°C for 10 s) is almost free of heating flavors. High-pasteurized milk and cream (e.g., 83°C for 10 s) have a flavor profile in which cooked flavor is caused mainly by hydrogen sulfide. It should be noted that volatile sulfur compounds that contribute to heat-induced flavors are formed mostly by thermal conversion of milk proteins (primarily proteins from the fat globule membrane).

Milk lipids are another source of heating flavors. Methyl ketones (2-alkanones) are formed upon thermal decarboxylation of $\beta$-keto fatty acids (liberated by hydrolysis of milk fat):

$$R{-}\overset{\overset{O}{\|}}{C}{-}CH_2{-}COOH \rightarrow R{-}\overset{\overset{O}{\|}}{C}{-}CH_3 + CO_2 \qquad (18.2)$$

Similarly, $\gamma$- and $\delta$-hydroxy fatty acids may produce the corresponding lactones:

$$R{-}\underset{\underset{OH}{|}}{CH}{-}CH_2{-}CH_2{-}CH_2{-}COOH \rightarrow \quad + H_2O \qquad (18.3)$$



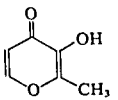
In UHT milk the typical flavor is caused chiefly by 2-alkanones, lactones, and sulfur compounds. Milk subjected to more intense heat treatment may develop a more complex heat-induced flavor. The typical sterilization flavor of high-heated milk (e.g., 10 min at 115°C) is mainly attributable to such compounds as maltol, iso-maltol, and certain furanones resulting from thermal conversion of sugars and from Maillard reactions.

**Maillard Reactions.** Maillard reactions make an important contribution to the formation of flavor compounds in milk and dairy products during heating, drying, and storage Lactose and milk proteins may undergo carbonyl-amino reactions of a very complex nature, involving (1) carbonyl-amino reaction, (2) rearrangement, (3) further amino reaction, and (4) degradation of amino sugars into flavor compounds and brown colors Examples of flavor compounds formed in this way are furanes (5-hydroxy methyl furfural, 4-hydroxy-2,5-dimethyl-3-[2H] furanone), pyrroles (N-substituted pyrrole aldehydes), pyrazines (dialkyl pyrazines), and many other classes of (heterocyclic) compounds containing S, N, and O In Table 18 3 some examples are given of aroma compounds formed by Maillard reactions and/or sugar caramelization and their flavor impact

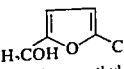
These aroma compounds may contribute to desirable flavors in food products such as coffee cream and evaporated milk However, other Maillard reactions, as a result of excessive heating or prolonged storage, may produce compounds that are undesirable at the concentration level in which they are present A typical off-flavor occurring in stored sterile concentrated milk and dried milk products is gluey flavor, which is caused mainly by *o*-amino acetophenone It probably is formed by degradation of tryptophan

**Light-Induced Flavor.** Light induced flavor can occur in milk and milk products upon exposure to (sun)light Riboflavin catalyzes the breakdown of methionine to 3-methyl thiopropanal Upon continued exposure methyl mercaptan and sulfides are formed It is evident that oxidation processes also are initiated by light radiation

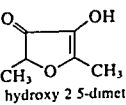
**Table 18.3. Examples of Aroma Compounds Formed by Maillard Reactions and/or Sugar Caramelization**



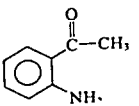
maltol
(caramel)



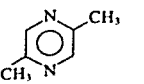
5 hydroxymethyl furfural
(malty)



4 hydroxy 2 5-dimethyl
3(2H) furanone (caramel)



o amino acetophenone
(gluey stale)



2,5-dimethylpyrazine
(roasted)

### 18.3.3. Off-Flavors Caused by Microorganisms and Enzymes

The growth of microorganisms in milk and dairy products easily may produce different kinds of flavor defects. Similar defects may be caused by the action of bacterial or milk enzymes.

**Unclean Flavor.** Unclean flavor may be the result of an increase in the concentration of dimethyl sulfide, as caused by rod-shaped Gram-negative psychrotrophic bacteria, which may develop at temperatures of 4°C or above.

**Fruity Flavor.** Fruity flavor results from ethyl esters of butyric, isovaleric, and caproic acid, which are produced by certain psychrotrophic organisms such as *Pseudomonas fragii*.

**Malty Flavor.** Malty flavor is caused mainly by 3-methyl butanal, 2-methyl butanal, and 2-methyl propanal, which result from growth of *Streptococcus lactis* var. *maltigenes*.

**Phenolic Flavor.** Phenotic flavor may develop occasionally in in-bottle sterilized milk because of contamination with spores of certain types of *Bacillus circulans*. Cresols probably are responsible for this defect.

**Bitter Flavor.** Bitter flavor occurs occasionally in UHT milk. This defect is caused by the breakdown of milk proteins by proteinases during prolonged storage. A sufficiently intensive heat treatment (more than 16 s at 142°C) may inactivate the milk proteinase (plasmin), but some bacterial proteinases will resist even very rigorous heat treatment (6 min at 142°C).

**Rancid Flavor.** Rancid flavor in milk and milk products is caused by liberation of lower fatty acids ($C_4$-$C_{12}$) by milk lipase or by bacterial lipases. In rancid milk the acidity of the milk fat (BDI test) exceeds 1.4 m*M* alkali per 100 g fat. In milk products of lower pH, such as cultured milk, buttermilk, and yogurt, the concentration of undissociated fatty acids is increased, which results in rancidity at much lower BDI values, probably 0.7. (Further details on problems caused by lipolysis are discussed in Section 5.5.)

It should be emphasized that rancidity in milk as well as unclean and fruity flavors are important problems in connection with present methods of bulk milk collection and prolonged storage of raw milk.

## 18.4. FLAVOR OF MILK PRODUCTS

### 18.4.1. Concentrated and Dried Milks

The flavor of conventionally sterilized and UHT-sterilized concentrated milk is primarily determined by the heat treatment during processing, which may

give rise to browning reactions These result in the formation of compounds that are similar to those found in UHT and high-heated milk Volatile compounds in sterilized concentrated milk are 2-alkanones, benzaldehyde, acetophenone, 2-furfural, furfuryl alcohol, hydroxymethyl furfural, naphthalene, $\delta$ decalactone, benzthiazole, and *o* amino acetophenone During the storage period stale flavors may develop from an increased concentration of a number of these compounds *o*-amino acetophenone seems to be the most relevant compound for this flavor defect at concentrations of 1 $\mu g\ kg^{-1}$ and above A rubbery aroma results from increased concentrations of benzthiazole Off-flavors from oxidation are observed less frequently

The concentration of 5-hydroxymethyl furfural can be used in estimating the intensity of the heat treatment that has been applied in the normal processing of concentrated milk

The flavor of dried low-fat milk protein products has been the subject of several studies The musty/stale flavor of lactic casein is caused mainly by *o*-amino acetophenone Several alkyl pyrazines have been found in edible spray-dried whey subjected to nonenzymatic browning

In the manufacture of whole milk powder, high temperature treatments usually are applied to ensure sufficient oxidation stability These treatments impart heat-induced flavors to whole milk powder, which are caused by 2-alkanones and lactones formed from lipid precursors and other products of browning reactions mentioned before A main problem in the storage of whole milk powder is presented by the oxidation of lipids, which leads to the formation of carbonyl compounds that may cause tallowy, metallic, oily, and other oxidation flavors A special case of lipid oxidation can occur in milk powder when drying air containing $\mu g\ kg^{-1}$ concentrations of ozone is used This may result in the formation of 6 *trans*-nonenal, which produces an oxidation flavor at 0 07 $\mu g\ kg^{-1}$ concentration and above

Coconut flavor (caused by $\delta$-lactones) and stale flavors may develop if the storage temperature and the water content of the milk powder are too high Steam deodorization of milk fat has been proposed with the purpose of removing lactones and 2-alkanones and also decomposing and removing their precursors This method has been used for the manufacture of foam spray-dried powder from deodorized milk fat and skim milk

### 18.4.2. Cultured Dairy Products

The characteristic aroma of cultured dairy products is mainly the result of the metabolism of the component lactic bacteria strains Its flavor elements are an acid taste, superimposed on a typical cultured flavor that depends on the type of mixed-strain starter used

The most important aroma compounds that contribute to the typical flavor of lactic starter cultures and cultured buttermilk are diacetyl, acetaldehyde, dimethyl sulfide, acetic acid, and lactic acid Further details are given in Table 18 4

**Table 18.4. Principal Volatile Compounds Isolated from Starter Cultures**

| Importance | Compound |
|---|---|
| Primary | diacetyl, acetaldehyde, dimethyl sulfide, acetic acid, lactic acid, $CO_2$ |
| Secondary | aldehydes: $C_4$, $C_5$, $C_8$, $2MeC_3$, $3MeC_4$<br>2-alkanones: $C_3$, $C_4$, $C_5$, $C_6$, $C_7$, $C_9$, $C_{11}$<br>alkanols: $C_1$, $C_2$, $C_4$, $C_5$, $2MeC_3$, $3MeC_4$<br>methyl esters: $C_2$, $C_4$, $C_6$, $C_7$, $C_8$, $C_{10}$, $C_{12}$<br>ethyl esters: $C_1$, $C_2$, $C_4$, $C_6$, $C_8$, $C_{10}$, $C_{12}$<br>δ-lactones: $C_8$, $C_{10}$<br>miscellaneous: $H_2S$, methanethiol<br>2-phenylethanal,<br>2-phenylethanol |

It is the balance among the various aroma compounds and $CO_2$ (in itself not a flavor component but still important) produced by the starter culture, in combination with the aroma compounds originally present in the milk or cream, that determines the acceptability of the aroma. For this reason the composition of the mixed-strain starter may be responsible for a pleasant balanced flavor or for certain off-flavors. Green flavor or yogurt flavor is caused by acetaldehyde, which is produced if excessive numbers of *Streptococcus lactis* or *S. diacetylactis* are present in relation to the *Leuconostoc cremoris/lactis* population. The ratio of diacetyl to acetaldehyde is approximately 4:1 in desirably flavored cultured products; ratios of 3:1 or lower result in yogurt flavor.

Examples of other flavor defects that may occur in cultured dairy products and that are caused by lactic bacteria are malty and fruity flavors.

In cultured dairy products containing lipids (particularly phospholipids in buttermilk) oxidation products contribute significantly to the flavor. Examples are 1-penten-3-ol and 1-penten-3-one found in oxidized buttermilk. These compounds and other oxidation products, if present in low concentrations, may contribute to desirable flavors. Higher concentrations, however, lead to oxidation flavors such as metallic.

Yogurt is produced by incubation of heated milk with a culture of *Lactobacillus bulgaricus* and *S. thermophilus*. Compounds identified in yogurt aroma are given in Table 18.5. The strongest contribution is given by acetaldehyde, which is present in amounts of 13–16 $\mu g \cdot kg^{-1}$ in yogurt of good flavor quality.

### 18.4.3. Cream and Butter

The flavor of cream and butter is primarily determined by compounds that are present in the lipid fraction of milk or are formed from precursors during

**Table 18.5. Principal Volatile Compounds Isolated from Yogurt**

| Type | Compound |
|---|---|
| Alkanals | $C_2$, $2MeC_3$ |
| Alkanols | $C_2$, $C_{1-3}$ |
| 2-alkanones | $C_3$, $C_4$, $C_5\Delta^3$, $C_6$, $C_7$, $C_9$, $C_{11}$ |
| Fatty acids | $C_1$, $C_2$, $C_4$, $C_6$, $C_8$, $C_{10}$ |
| Ethyl esters | $C_1$, $C_2$ |
| $\gamma$-lactones | $C_5$ |
| $\delta$-lactones | $C_6$, $C_8$, $C_{13}$ |
| Miscellaneous | diacetyl, pentanedione-2,3, acetyl methylcarbinol, 3-hydroxypentanone-2, 2-hydroxypentanone-3, dimethyl sulfide.<br>benzaldehyde, benzylalcohol, methylbenzoate.<br>2-phenylethanal.<br>furfural, furfurylalcohol, 5-methylfurfural, 2,5-dimethylfuran, 2-pentylfuran, furyl methyl ketone, furyl ethyl ketone, furyl-2-propanal-3 |

processing. The significance of lactones for the flavor of milk fat and sweet-cream butter is well established, but several other compounds also contribute.

The principal aroma compounds isolated from sweet-cream butter are listed in Table 18.6. Alkanoic acids ($C_{10,12}$), $\delta$-lactones ($C_{8,10,12}$), indole, and skatole are present in quantities high enough to contribute to the flavor in the range of organoleptically desirable levels, whereas phenol, *m*- and *p*-

**Table 18.6. Principal Volatile Compounds Isolated from Butter**

| Type | Compound |
|---|---|
| Alkanals | $C_{1-12}$, $2MeC_3$, $3MeC_4$ |
| Alkenals | $C_5\Delta^2$-$C_{11}\Delta^2$ |
| 2-alkanones | $C_3$-$C_{15}$ |
| Other carbonyls | $C_7$-3one, diacetyl, acetoin, benzaldehyde, 2-phenylethanal, phenylpropandione |
| Lactones | $\delta C_{6-18}$, $\gamma C_{8-16}$, $\gamma\Delta^9C_{12}$, $\delta\Delta^9C_{14}$, bovolide |
| Alkanols | $C_{1-10}$ |
| Fatty acids | $C_2$, $C_4$, $C_{1-4}$, $C_{1-5}$, $C_6$, $C_8$, $C_{1-8}$, $C_{10}$, $C_{12}$ |
| Methyl esters | $C_1$, $C_2$, $C_{10}$, methylbenzoate |
| Ethyl esters | $C_1$, $C_2$, $C_4$, $C_6$, $C_7$, $C_8$, $C_{10}$, $C_{12}$ |
| Phenols | phenol, *m*-cresol, *p*-cresol, *o*-methoxyphenol |
| Miscellaneous | dimethyl sulfide, indole, skatole, $H_2S$, benzthiazole |

cresol, and *o*-methoxy phenol are of borderline significance. Other components reported to be important to the flavor of butter are dimethyl sulfide and 4-*cis*-heptenal. It should be noted that flavor and taste elements (e.g., salt) are better perceived if the moisture droplets in butter are larger.

The flavor of cultured-cream butter is composed of aroma compounds also present in sweet-cream butter and of others present in starter culture. Both products already have been discussed. Diacetyl seems to be the most important component which, together with acidic constituents (mainly lactic and acetic acids), sweet-cream butter constituents, and dimethyl sulfide (which balances the diacetyl flavor), creates a fresh, mild, full creamy flavor.

In Table 18.7 a composition is given that produces a flavor similar to that of ripened-cream butter, when added to sweet-cream butter.

The flavor of cream differs from that of sweet-cream butter mainly in its higher quantity of constituents from the aqueous phase of milk and the fat globule membrane. Whipping of the cream may give a slight increase in oxidation products, which (if present in very low concentrations) improves the flavor. 4-*cis*-heptenal in particular, if present in $\mu g \cdot kg^{-1}$ quantities, contributes to the full flavor of cream. Hydrogen sulfide, dimethyl sulfide, lactones, and other compounds present in sweet-cream butter also contribute.

A number of flavor defects, such as rancid, unclean, malty, or yeasty, which were mentioned before and which are caused by enzymatic or microbial spoilage, also may occur in butter and cream. Yogurt flavor may occur in butter if the concentration of acetaldehyde is 0.3 ppm or more. Cooked flavor caused by hydrogen sulfide and resulting from heat treatments to which the cream is subjected can be prevented largely by cooling the milk (2 h at 5°C) before separating the cream, and by pasteurization at lower intensity.

A major cause of flavor defects in cream and butter is the susceptibility of these products to oxidative deterioration. Compounds contributing to

**Table 18.7. Formulation for Cultured-Butter Flavor (mg/kg added to sweet cream butter)**

| | | | |
|---|---|---|---|
| Diacetyl | 4. | γ-Decalactone | 3. |
| 3-Methylbutanal | 0.01 | δ-Decalactone | 3.6 |
| 4-*cis*-Heptanal | 0.006 | δ-Dodecalactone | 6.9 |
| 2-Phenylethanal | 0.002 | $H_2S$ | 0.01 |
| Acetic acid | 57. | Methyl mercaptan | 0.01 |
| Valeric acid | 0.15 | Dimethyl sulfide | 0.06 |
| Phenol | 0.01 | Indole | 0.006 |
| *p*-Cresol | 0.005 | Skatole | 0.67 |
| Guaiacol | 0.002 | Sodium glutamate | 1. |
| Ethyl butyrate | 0.002 | Lactic acid to pH 4.6 | |

either desirable flavors or to typical oxidation defects, according to their concentration, have been discussed before. (See Table 18.2.)

### 18.4.4. Cheese

There are many hundreds of cheese varieties, but extensive flavor studies have been made of only a limited number. The discussion on cheese flavors, therefore, will be restricted to the latter.

Most types of cheese have something like a basic cheese flavor in common, which is caused by salt, lactic acid, protein degradation products, and (often) lipid degradation products. The balance among these compounds is very different for different types of cheese. Also, $CO_2$ must be considered to contribute to the overall flavor of cheese.

**Cheddar Cheese.** Free fatty acids (ffa) are constituents of Cheddar cheese flavor. However, any correlation between Cheddar flavor and the ffa content is difficult to establish. At any rate, milk fat seems to be essential to the development of Cheddar flavor.

Many aroma compounds in Cheddar cheese have been identified, but information on the flavor significance of these compounds is limited. From recent work it is clear that only cheese that has been made from whole milk with a starter culture develops a normal Cheddar flavor. The background flavor is caused mainly by peptide, amino acids, and other products of protein breakdown, whereas hydrogen sulfide, methanethiol, dimethyl sulfide, and diacetyl are found in the volatile fraction. Probably methanethiol is essential to Cheddar cheese aroma. The presence of hydrogen sulfide is beneficial. Volatile acids appear not to contribute to the aroma, but they may affect the taste of the cheese. While 2-alkanones and lactones may contribute to some extent to flavor, on their own they do not impart a typical Cheddar flavor to cheese. A good Cheddar cheese flavor seems to be related to the balance between the free fatty acids and the sulfur compounds.

**Blue-Veined Cheese.** Although different types of blue cheese, such as Roquefort, Stilton, and Gorgonzola, differ in their delicate flavor, they have a basic flavor in common that results particularly from free fatty acids, methyl ketones, and 2-alkanols. Other compounds identified are $\alpha$-keto acids, aldehydes, ethyl esters, and amines. Low concentrations of 1-octen-3-ol also have been detected. A list of principal volatile compounds found in blue cheese is given in Table 18.8. The present knowledge makes it possible to formulate synthetic blue-cheese flavor or to produce it by submerged fermentation of *Penicillium roqueforti*.

**Camembert.** The flavor of Camembert comes partly from the white mold (particularly *P. candidum*) growing on its surface. Flavor compounds are listed in Table 18.9. A major difference in flavor between Camembert and

**Table 18.8. Principal Volatile Compounds Found in Blue Cheese**

| | |
|---|---|
| Alkanoic acids | $C_2$, $C_4$, $2MeC_3$, $2MeC_4$, $C_6$, $C_8$, $C_{10}$, $C_{12}$ |
| 2-alkanones | $C_3$, $C_5$, $C_7$, $C_9$, $C_{11}$ |
| 2-alkanols | $C_5$, $C_{7-5}$, $C_7$, $C_9$ |
| Methyl esters | $C_4$, $C_6$, $C_8$, $C_{10}$, $C_{12}$ |
| Ethyl esters | $C_1$, $C_2$, $C_4$, $C_6$, $C_8$, $C_{10}$ |
| Alkanals | $C_2$, $C_3$, $C_4$, $C_{7-4}$, $C_{7-5}$, 2-phenylethanal |
| 1-alkanols | $C_4$, $2MeC_4$, $3MeC_4$, 2-phenylethanol |
| Miscellaneous | keto acids, amines $C_1$, $C_2$, $C_4$, $C_{7-4}$, $C_{7-5}$ dialkylamines $C_1$, $C_2$, $C_3$, $C_4$ |

blue cheese seems to be that the concentration of 1-octen-3-ol in the former cheese is higher. Blue-cheese flavor can be changed into a typical Camembert flavor simply by adding a sufficient quantity of 1-octen-3-ol.

**Limburger and Trappist Cheese.** These types of cheese have a surface flora consisting of several species of yeasts and bacteria (particularly *Brevibacterium linens*). The flora contribute to the formation of a very pronounced flavor, which is caused by hydrogen sulfide and particularly methanethiol.

**Swiss cheese.** Volatile fatty acids, particularly propionic acid, are important constituents of Swiss cheese flavor, but many other compounds also

**Table 18.9. Principal Volatile Compounds Found in Camembert Cheese**

| | |
|---|---|
| 1-alkanols | $C_2$, $C_3$, $C_4$, $C_6$, $2MeC_3$, $3MeC_4$, 1-octen-3-ol 2-phenylethanol |
| 2-alkanols | $C_4$, $C_5$, $C_6$, $C_7$, $C_9$, $C_{11}$ |
| 2-alkanones | $C_4$, $C_5$, $C_6$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $\Delta C_9$, $\Delta C_{11}$ |
| Alkanals | $C_6$, $C_7$, $C_9$, $2MeC_4$, $3MeC_4$ |
| Ethyl esters | $C_2$, $C_4$, $C_6$, $C_8$, $C_{10}$ |
| Other esters | methyl cinnamate, 2-phenylethylacetate, 2-phenylethylbutyrate |
| Phenols | phenol, *p*-cresol |
| Lactones | $\gamma C_9$, $\delta C_{10}$, $\delta C_{12}$ |
| Sulfur compounds | dimethyl sulfide, 2,4-dithiapentane, 2,4,-5-trithiahexane, 3-methylthiopropanol |
| Anisoles | anisole, 4-methylanisole, 2,4-dimethylanisole |
| Miscellaneous | indole |

contribute to it. Probably keto-acids, which were found in the fluid in the holes of the cheese, also play a role. More recently it was found that monoamines and alkyl pyrazines also may be important.

**Gouda Cheese.** The knowledge of the nature of the flavor compounds in Gouda cheese is limited at present. However, it is clear that the aroma results from the breakdown of lipids, casein, lactose, and citric acid. Anethole, 2,4-dithiopentane, and several alkyl pyrazines also were found in the aroma of Gouda cheese. It is the correct balance among the various flavor substances that creates a desirable Gouda cheese flavor. Any imbalance may cause flavor defects (e.g., excessive formation of free fatty acids may lead to rancidity, and certain peptides may cause a bitter taste). Other flavor defects may be caused by excessive formation of esters (fruity flavors), *p*-cresol (phenolic flavor), and 4-mercapto-4-methyl-pentanone-2 (ribes or catty taints).

## *Suggested Literature*


Aspects of sensory evaluation are discussed in

M A Amerine, R M Pangborn, and E B Roessner, *Principles of Sensory Evaluation of Food* (New York: Academic Press, 1965)

J A Nelson and G M Trout, *Judging Dairy Products* (Madison: Olsen, 1965)

Methods of analysis of flavor compounds are given in

R Teranishi, R A Flath, and H Sugisawa, eds., *Flavor Research: Recent Advances* (New York: Dekker 1982)

Reviews on aroma compounds in milk and dairy products and mechanisms of formation, including many literature references to the subjects discussed in this chapter, are

H T. Badings and R Neeter, "Recent Advances in the Study of Aroma Compounds of Milk and Dairy Products," *Neth. Milk Dairy J.* 34 (1980) 9

D A Forss, "Mechanisms of Formation of Aroma Compounds in Milk and Milk Products," *J. Dairy Res.* 46 (1979) 691

Specific subjects on dairy flavors:

Lipids and flavors: See references to Chapter 5

Oxidation and flavors: H T Badings, *Neth. Milk Dairy J.* 24 (1970) 147

Fermented dairy products: W E Sandine and P. R Elliker, *J. Agric. Food Chem.* 19 (1970) 557.

Dried milk products: N J Walker, *Proc. Jubilee Congr. Dairy Sci. Technol. New Zealand* (1977) 49

# 19

# Nutritive Value

## 19.1. GENERAL ASPECTS

The nutritive value of a foodstuff can be assessed by (1) the energy it supplies, (2) its content of essential nutrients (fatty acids, amino acids, minerals, and vitamins), (3) the digestibility and absorbability of its nutrients, and (4) its content of harmful allergens, protease inhibitors, and toxins. Nutritive quality can be evaluated by analysis for constituents or by feeding trials to determine the extent to which growth is supported, nutrients are retained, or health is maintained.

The primary emphasis in this chapter is on the nutritive value of milk for humans. Some societies rely heavily on milk to supply certain nutrients to adults, and milk frequently is the sole or major component of the diet of human infants. Thus, it is natural that much interest has centered on the ability of cow's (and to a lesser extent goat's) milk to supply the nutritional needs of human adults and infants. The principal approach has been to relate composition of milk to nutrient requirements. Only a few direct feeding trials of humans with milk have been made. Nutrient requirements have been established in several countries (see, for example, the Recommended Dietary Allowances of the Food and Nutrition Board, National Academy of Sciences, U.S.A.). These standards probably have been set somewhat high to include a safety factor and to allow for natural variation in the requirements of individuals.

Gross energy supplied by milk can be calculated from its fat, protein, and lactose contents. On complete combustion these yield 38.5, ~25.0, and 17.3 $kJ \cdot g^{-1}$, respectively. The value for milk fat is somewhat lower than the average taken for dietary fats (~39.3) because of its high content of short-chain fatty acids. The value for protein is somewhat uncertain, partly because of confusion about crude and true protein. Taking into account that digestibility is less than complete and that part of the protein is excreted as urea and some other metabolites, the metabolically available energy is about 37.0,

17.0, and 16.8 kJ·g$^{-1}$ (8.9, 4.1, and 4.0 kcal·g$^{-1}$) for fat, protein, and lactose, respectively. To this may be added ~15 kJ·g$^{-1}$ for lactate and ~10 kJ·g$^{-1}$ for citrate. Thus, if $F$, $P$, and $L$ are contents in % (w/w), available gross energy of milk (in kJ·kg$^{-1}$) is approximately

$$E = 370\,F + 170\,P + 168\,L + 18 \qquad (19.1)$$

Using the compositional data of Table 9.1, human, cow's and goat's milk are calculated to have similar energy contents of 2.8–3.0 MJ·kg$^{-1}$ (670–720 kcal·kg$^{-1}$). In human milk, fat, protein, and lactose account for about 55%, 5%, and 40% of the energy, but in cow's milk they furnish about 52%, 20%, and 28%, respectively. Growth rates of human infants have been found to be approximately equal when human, cow's, or goat's milk or suitable vegetable diets are fed in isocaloric quantities.

The nutritional adequacy of milk for humans can be presented as a profile relating the content of various constituents to requirements and using energy as a common denominator; the resulting quotient is designated nutrient density. Thus, for example, human infants require about 0.43 g protein per 100 kJ. Cow's milk, with 3.2 g protein and 275 kJ per 100 g, furnishes 1.16 g protein per 100 kJ. Consequently, it provides 1.16/0.43 or 2.7 times the amount of protein needed in relation to energy. The profile shown in Table 19.1 was calculated in this way. Obviously, if the requirements are set high, as they probably are in most cases, such profiles underestimate the ability of milk to meet requirements. Comparison of the profiles of human and cow's milk in meeting the requirements of human infants (Table 19.1) indicates the great excess in protein, calcium, and phosphorus in cow's milk. For adults milk furnishes important excesses of protein, thiamin, riboflavin, and calcium over energy but is deficient in iron and vitamin C. Concentrations of milk nutrients and dietary requirements are tabulated in the appendix, Tables A.1, A.14, and A.25, while Table A.15 gives data on milk products.

## 19.2. CARBOHYDRATES

Lactose functions chiefly as a source of energy, furnishing 16·8 kJ·g$^{-1}$. It is not required as a source of galactose because animals have more than sufficient capacity to epimerize glucose to galactose. Like other disaccharides, lactose is not transported across the intestinal membranes unless hydrolyzed to its monosaccharide components; glucose is taken up more readily than galactose. Lactose thus is more slowly metabolized than, say, glucose, which prevents the occurrence of undesirable peak concentrations of glucose in the blood after drinking milk.

$\beta$-galactosidase (lactase) specific for the $\beta$-1,4-linkage in lactose is found in the brush border mucosal cells of the intestine. However, in some individuals this enzyme is absent or present in only low concentrations. Lack

**Table 19.1. Nutrients in Fresh Milk (~350 g) in Relation to Energy**

| | | Content | | Requirement[a] per 1000 kJ | Nutrient Density | |
|---|---|---|---|---|---|---|
| | Unit | Cow A | Human B | C | A/C | B/C |
| Energy | (kJ) | 1000. | 1000. | | 1.0 | 1.0 |
| Protein | (g) | 11.6 | 3.0 | 4.3 | 2.7 | 0.7 |
| Vitamin A | (RE)[b] | 157. | 333. | 100. | 1.6 | 3.3 |
| Vitamin D | ($\mu$g) | 0.2 | 0.2 | 2.5 | 0.08 | 0.08 |
| Vitamin E | (mg) | 0.35 | 2.20 | 1.0 | 0.35 | 2.20 |
| Ascorbic acid | (mg) | 7.6 | 14.3 | 8.8 | 0.9 | 1.6 |
| Thiamin, $B_1$ | (mg) | 0.16 | 0.05 | 0.12 | 1.3 | 0.4 |
| Riboflavin, $B_2$ | (mg) | 0.64 | 0.12 | 0.15 | 4.3 | 0.8 |
| Niacin | (NE)[c] | 2.4 | 0.6 | 2.0 | 1.2 | 0.3 |
| Vitamin $B_6$ | (mg) | 0.23 | 0.03 | 0.15 | 1.5 | 0.2 |
| Folic acid | ($\mu$g) | 18. | 16. | 11. | 1.6 | 1.4 |
| Vitamin $B_{12}$ | ($\mu$g | 1.56 | 0.10 | 0.38 | 4.1 | 0.3 |
| Calcium | (mg) | 450. | 100. | 135. | 3.3 | 0.7 |
| Magnesium | (mg) | 47. | 13. | 17.50 | 2.7 | 0.7 |
| Iron | (mg) | 0.16 | 0.33 | 3.80 | 0.04 | 0.08 |
| Zinc | (mg) | 1.4 | 0.83 | 1.2 | 1.2 | 0.7 |
| Phosphorus | (mg) | 360. | 50. | 90. | 4.0 | 0.6 |
| Iodine | ($\mu$g) | 15.6 | 33.3 | 10. | 1.6 | 3.3 |

[a]For infant one-half to one year weighing 9.0 kg and requiring about 4,000 kJ per day.
[b]Retinol equivalent ($\mu$g retinol + $\mu$g carotene/6)
[c]Niacin equivalent (mg niacin + mg Trp/60; Trp corrected for 189 mg/day required by 9 kg infant).

of lactase allows lactose to pass into the large intestine, where it is subject to degradation by microorganisms, some of which produce gas. Furthermore, it partly prevents water from being drawn osmotically out of the large intestine. Thus, abdominal cramps, bloating, flatulence, and diarrhea may result. The phenomenon is termed lactose intolerance. It can be assessed by (1) measuring changes in blood sugar with time after ingestion of a dose of lactose, (2) measuring $^{14}CO_2$ in expired air after ingestion of a dose of [$^{14}$C]-lactose, (3) assaying for lactase in a specimen of intestinal tissue taken by biopsy, or (4) radiological examination of the intestinal surface after ingestion of barium. The doses given in methods 1 and 2 are generally about 1 g lactose per kilogram of body weight. Such a dose, however, which is equivalent for an adult to 1.0–1.5 liter of milk at a single meal, likely classifies

as intolerant many individuals with sufficient lactase to handle more usual loads of lactose.

Lactase deficiency may result from gastroenteritis. Microorganisms attacking the brush border membrane of the small intestine may so alter it that its lactase activity is impaired. Lactase deficiency also may result from genetic defects. A rare congenital lactase deficiency has been observed in a few infants. Much more common is a pattern of satisfactory lactose metabolism in infancy followed by loss of lactase between ages one and four. The incidence of lactose intolerance varies greatly among ethnic groups. Descendants of groups in which adults have consumed milk for generations have much lower incidence than those from nondairy societies. For example, incidences of 10% and 70% have been reported in American white and black adults, respectively. Whether lactase is induced or constitutive has not been established clearly. No clearcut evidence has been adduced that the enzyme can be induced or its loss during maturation prevented by consuming large quantities of milk. Present thinking favors the idea that the loss of lactase during maturation is controlled genetically and that the low incidence of lactose intolerance in some ethnic groups has resulted from a selective process. Loss of lactase activity during maturation also occurs in several other species of mammals. Generally, however, the level does not fall so low that the animal cannot metabolize considerable lactose.

Another genetic defect in lactose metabolism is galactosemia, which is caused by hereditary lack of the liver enzyme, galactose-1-phosphate uridyl transferase, which catalyzes one of the steps (equation 19.3) in the metabolism of galactose. Three steps in the pathway are:

$$\text{Gal} + \text{ATP} \xrightarrow{\text{kinase}} \text{Gal-1-P} + \text{ADP} \tag{19.2}$$

$$\text{Gal-1-P} + \text{UDP-Glc} \xrightarrow{\text{transferase}} \text{UDP-Gal} + \text{Glc-1-P} \tag{19.3}$$

$$\text{UDP-Gal} \xrightleftharpoons{\text{epimerase}} \text{UDP-Glc} \tag{19.4}$$

In the absence of the transferase, galactose, galactose-1-phosphate, and galactitol accumulate in the blood and tissues when galactose-containing foods are ingested. A variety of symptoms occur in galactosemia, including vomiting, diarrhea, failure to thrive, eye cataracts, and mental retardation. Some of the symptoms are considered to be caused by galactitol, which is formed by reduction of galactose. The only known cure is complete elimination of galactose from the diet. Familial studies have established galactosemia as an autosomal–recessive disease. It is exhibited by homozygotes only. Estimates of its frequency are one per 20,000–70,000 live births. Another, very rare type of galactosemia results from a genetic defect in which the kinase catalyzing Reaction 19.2 is lacking. Galactose and galactitol, but not galactose-1-P, accumulate in the tissues.

Lactose enhances the passage of certain metallic elements across the

brush border membrane of the intestine, specifically the ileum. Monosaccharides and disaccharides that are hydrolyzed before reaching the small intestine do not enhance absorption. The enhancing effect of lactose has been noted in both in vivo and in vitro experiments, mostly with laboratory rats and gut preparations from them. The effect has been observed with Ca, Ba, Sr, Rb, Mg, Fe, Co, Zn, and Pb. It is considered to be an important supplement to vitamin D in facilitating absorption of Ca. The mechanism by which lactose operates in this phenomenon is not known but probably involves an alteration in structure of the brush border membrane rather than formation of lactose–metal complexes.

One outstanding difference between human and cow's milk is the much greater content of oligosaccharides in the former. About thirty of them have been isolated from human milk, ranging from three to fourteen saccharide units per molecule. All contain reducing terminal glucose; the other monosaccharide components are galactose, fucose, N-acetyl glucose, N-acetyl galactose, and N-acetyl neuraminic acid. The total concentration of these oligosaccharides is on the order of 10 $g \cdot kg^{-1}$ in midlactation milk and 25 $g \cdot kg^{-1}$ in colostrum. By contrast cow's milk contains only about 1 g of oligosaccharides per kilogram (some listed in Table A.9). Several of the human milk oligosaccharides, especially certain N-containing tetra- and pentasaccharides, promote the growth of some strains of *Bifidobacterium bifidum* (*Lactobacillus bifidus*). They often are cited as possible controllers of the intestinal flora of breastfed infants.

## 19.3. MINERALS

Mineral elements have many roles in animals. Ca and P perform structural functions in bone and teeth. Many enzymes require specific metallic activators including Zn, Mg, Fe, Cu, Mn, or Mo. Co is an essential part of vitamin $B_{12}$, and Fe of hemoglobin. Minerals are important buffer constituents and contribute strongly to maintenance of pH, ionic strength, and osmotic pressure in body fluids and tissues. Phosphate esters and anhydrides (such as ATP) participate in and are essential to the processes involved in energy metabolism.

Milk supplies virtually all of the minerals, major and minor, required by humans. Concentrations of minerals in human and cow's milks and human daily requirements are given in Tables A.1 and A.25. Cow's milk furnishes a large portion of the total Ca consumed in some countries (as much as 75% of the total in the "American diet"). It has a great excess of Ca and phosphate in relation to energy for human infants. (See Table 19.1.) Absorption of the Ca of milk in the intestine is very good; it is favored by lactose, as already mentioned, and by vitamin D. Phosphate absorption is correlated with that of Ca. The fairly high ratio of Ca to P in milk (about 0.9 on a molar basis, including organic P) is considered to be advantageous, especially in elderly people for the prevention of excessive osteoporosis.

Milk is poor in Fe and neither cow's nor human milk furnish enough for human infants Fortunately, infants have a considerable liver store of Fe at birth Intestinal absorption of Fe is greater from human than from cow's milk although variations among individual infants are large The superior absorption may be related to the higher contents of lactoferrin, lactose, and ascorbate, and the lower phosphate content of human milk Most of the Zn in human milk is bound to a low molecular weight ligand, probably citrate Such binding favors its absorption from the intestine Zn in cow's milk is predominantly bound to calcium phosphate in the casein micelles, it is released by acidification as in the stomach

## 19.4. LIPIDS

Dietary lipids primarily serve as a source of energy Milk lipids average 37 kJ $g^{-1}$, individual triglycerides vary in energy "content" with length of fatty acid chains Milk fat is nearly always fully liquid at body temperature (37°C), which is a prerequisite for its digestibility Milk lipids are a source of certain essential fatty acids, and they carry fat-soluble vitamins Milk lipids are important contributors to both desirable and undesirable flavors in milk and dairy products (See Chapter 18 )

In the animal liquid triacylglycerols are hydrolyzed by pancreatic lipases in the intestine to yield mostly 2-monoacylglycerols, a little 1,2-diacylglycerols, and fatty acid soaps This process is facilitated by bile salts that aid in finely emulsifying the oil (thus enlarging its surface area) and promote the access of lipase to the oil surface The resulting acylglycerols, soaps, and bile salts enter the intestinal mucosal membrane There resynthesis to triacylglycerols occurs, and small oil droplets called chylomicrons are formed, which proceed via the lymph system to the blood circulation The digestion and absorption of triacylglycerols varies with fatty acid composition and with the pattern of arrangement of fatty acid residues in the molecule Long-chain saturated fatty acids (16 0 and 18 0) form insoluble soaps with Ca and may not be completely absorbed Short-chain acids (6 0–10 0) are not efficiently reesterified in the mucosa and are carried as such to the liver for metabolism Human milk fat is better absorbed by very young infants than is cow's milk fat This difference may be caused in part by differences in fatty acid composition (see Table 19 2) but probably largely results from difference of arrangement of the fatty acids in the triacylglycerols In human milk fat palmitic acid is esterified primarily in the 2 position (which implies formation of 2-monopalmitate that is readily absorbed) whereas in cow's milk fat it is distributed more nearly equally in all three positions (giving insoluble soaps) The digestive and absorptive processes thus are favored when palmitic and stearic acids are in the 2-position

It has been suggested by some that differences in fat globule size influence rates of digestion and absorption In particular, goat's milk fat has been alleged to be more rapidly digested and absorbed because of its smaller fat

**Table 19.2. Approximate Fatty Acid Composition (% w/w) of Milk Fats**

| Acid Residue | Cow's Milk | | Human Milk | |
|---|---|---|---|---|
| | Winter | Summer | Range | Mean |
| **Short Chain** | | | | |
| Butyric | 3.6 | 3.1 | <1 | |
| Caproic | 2.2 | 1.7 | | |
| Caprylic | 1.4 | 1.0 | | |
| Capric | 2.9 | 2.0 | 0.3–2.0 | 1.0 |
| **Long Chain, Saturated** | | | | |
| Lauric | 3.4 | 2.4 | 2.0–8.0 | 5.0 |
| Myristic | 12.0 | 10.0 | 4.0–17.0 | 8.0 |
| Palmitic | 31.0 | 24.0 | 20.0–28.0 | 24.0 |
| Stearic | 10.0 | 13.0 | 3.0–10.0 | 8.0 |
| Arachidic | 0.2–1.2 | | 0.5–1.3 | 1.0 |
| **Monounsaturated** | | | | |
| Palmitoleic | 3.0 | 2.7 | 1.0–6.0 | 3.0 |
| Oleic | 23.0 | 31.0 | 28.0–43.0 | 35.0 |
| **Polyunsaturated** | | | | |
| Linoleic | 2.0 | 2.2 | 4.0–20.0 | 10.0 |
| Linolenic | 1.2 | 2.4 | 0.5–3.0 | 1.0 |
| Arachidonic | 0.1–1.0 | | 0.5–3.0 | 1.0 |

*Note:* Wide variation occurs according to nutrition and genetic factors.

globules. There is no experimental evidence supporting such an allegation. Fat globules are not absorbed as such, and undoubtedly the size of the original globule has little influence on the rate of hydrolysis in the presence of the emulsifying bile salts. Some experimental work with homogenized milk failed to demonstrate a more rapid rate of fat digestion and absorption than in unhomogenized milk.

Certain fatty acids not synthesized by animals are essential precursors of prostaglandins and thromboxanes. These include the polyunsaturated acids linoleic, 18:2-Δ9,12 *cis cis*, and arachidonic 20:4-Δ5,8,11,14 all *cis*. In general it is considered that at least 1% of the total energy of the diet should be supplied by such acids, and 4% is perhaps preferable for human infants. Table 19.2 shows that the contents of these acids are much greater in human than in cow's milk fat. The linoleic acid in human milk fat accounts for 4–5% of the energy in the milk; cow's milk fat contains only enough to account for about 1% of the total energy. Neither fat contains much arachidonic acid.

Thus, it is sometimes recommended that cow's milk formulas for infants be supplemented with a source of 18 2 acid such as corn oil Human milk contains small concentrations of prostaglandins but cow's milk appears to have none

Much interest has centered on dietary lipids in relation to atherosclerosis and coronary heart disease Among the several factors that seem to be correlated with high incidence of this condition is elevated cholesterol content of the blood serum Part of the cholesterol in animals comes from the diet, but a larger part is synthesized internally Dietary long chain saturated fatty acids ($> C_{10}$) tend to elevate and polyunsaturated fatty acids to lower cholesterol levels Cow's milk fat contains 300–350 mg cholesterol per 100 g fat and has a low ratio of polyunsaturated to long-chain saturated fatty acids of about 0 1 For these reasons, restriction or exclusion of milk fat from the diet sometimes has been recommended The fact is, however, that milk itself (not milk fat) is hypocholesteremic, it depresses serum cholesterol levels The factor(s) in the nonfat portion responsible for this effect and the mechanism by which it is achieved are not known

## 19.5. PROTEINS

The nutritive value of milk proteins depends primarily on their content of the nine essential amino acids that humans cannot synthesize Cow's and human milks differ markedly in the proportions of the various proteins that they contain (See Table 19 3 ) Nevertheless, the mixture of proteins of either

**Table 19.3. Approximate Protein Composition of Cow's Milk and Human Milk**

| | Concentration (g kg $^{-1}$) | | | |
|---|---|---|---|---|
| Protein | Cow | | Human | |
| Casein[a] | 26 0 | | 3 3 | |
| Fat globule membrane | 0 4 | | 0 3[a] | |
| α lactalbumin | 1 2 | | 1 5 | |
| β-lactoglobulin | 3 2 | | 0 0 | |
| Blood serum albumin | 0 4 | | 0 4 | |
| Immunoglobulins | 0 7 | 6 3 | 1 4 | 5 2 |
| Lactoferrin | <0 1 | | 1 5 | |
| Lysozyme | ~10$^{-4}$ | | 0 4 | |
| Miscellaneous | 0 8 | | ? | |
| Total | 33 0 | | 9 0 | |

[a]Casein composition is also different human milk having predominantly β-casein

species adequately fulfills the pattern of essential amino acid requirements for human infants. (See Table 19.4.) The digestibility of milk proteins and absorption of their amino acids is high. Biological indices sometimes used are:

1. Biological value (BV), the percentage of absorbed N retained in the body.
2. Coefficient of digestibility (TD) estimated from fecal N loss with and without protein in the diet.
3. Net protein utilization (NPU), a measure of the overall utilization of ingested N. NPU = BV × TD.
4. Protein efficiency ratio (PER), the ratio of weight gain of a growing animal to its protein intake.

Different authors have somewhat different results for the indices of milk proteins, partly because the indices depend to some extent on total protein and energy intake. Most agree on a coefficient of digestibility of at least 0.96, if it concerns fresh milk (intensive heating and long storage may somewhat decrease digestibility, partly because of Maillard reactions). Estimates for cow's casein are BV ≈ 0.80 and PER ≈ 2.5; whey protein BV = 0.85–0.90, PER ≈ 3.0; and whole protein BV ≥ 0.9, PER 3.2–3.4. The mixture of casein

**Table 19.4. Essential Amino Acids**

| Amino Acid | Quantity in Protein (mg·g$^{-1}$) | | | Daily Requirement (mg·kg$^{-1}$·day$^{-1}$) | |
|---|---|---|---|---|---|
| | Cow | Human | RDA[a] | Infant[b] | Adult |
| Histidine | 30 | 23 | 17 | 33 | ? |
| Isoleucine | 55 | 50 | 42 | 83 | 12 |
| Leucine | 100 | 91 | 70 | 135 | 16 |
| Lysine | 80 | 67 | 51 | 99 | 12 |
| Methionine | 28 | 14 | 26 | 49 | 10 |
| Cysteine | 9 | 18 | | | |
| Phenylalanine | 53 | 36 | 73 | 141 | 16 |
| Tyrosine | 44 | 28 | | | |
| Threonine | 48 | 42 | 35 | 68 | 8 |
| Tryptophan | 14 | 22 | 11 | 21 | 3 |
| Valine | 72 | 64 | 48 | 92 | 14 |

*Note:* Approximate contents in cow's and human milk protein and some dietary recommendations

[a]Estimated pattern of high-quality protein (NRC-NAS, U.S.).

[b]Four to six months old.

and whey protein has a higher quality than each one separately, because they are complementary in their concentrations of some essential amino acids; for instance, tyrosine plus phenylalanine are comparatively high in casein, cysteine plus methionine in whey protein. Protease inhibitors have been reported in colostrum of some species but apparently are not present in either cow's or human milk. That the balance of amino acids, digestibility, and absorbability for both cow's and human milk is satisfactory for human infants is attested by their high retention of N from either source.

It is of some importance for young infants to maintain their protein intake at a not too high level, to keep down the renal solute load. If a large portion of the absorbed amino acids is not retained for growth but metabolized, the urea content of the blood may become high and the kidney has difficulty in removing it. The higher the quality of the protein in the food, the smaller the protein intake can be.

Cow's milk forms a rather tough casein curd in the stomach. Gastric emptying and protein digestion are much slower than with human milk, which has so little casein that it scarcely curdles. Dilution of cow's milk with water or heat treatment sufficient to denature whey proteins, greatly softens the curd. The higher casein and calcium phosphate contents of cow's milk serve as effective buffers in the infant stomach and hinder the attainment of the proper pH (~3.75) necessary for optimal gastric digestion of the protein. Sour milk products are sometimes preferred in infant formulas to keep down stomach pH.

Some individual humans, particularly infants, exhibit allergic responses to ingested cow's milk proteins. Evidently they had been sensitized by previous exposure. The responses run the gamut from rather mild reactions such as rhinitis or diarrhea to dermatitis, asthma, and in a few cases anaphalaxis. Identification of milk as the causative agent is indicated by abatement of symptoms when it is withdrawn from the diet. Reliable epidemiological studies scarcely have been performed, and the incidence of milk allergies is far from certain; estimates range from 0.1–0.5% of the population. In a few controlled studies humans diagnosed as allergic to milk have been challenged by oral administration of purified milk proteins. When tested separately, casein, $\alpha$-lactalbumin, $\beta$-lactoglobulin, and serum albumin elicited reactions. Some studies have suggested the formation of new antigenic determinants by reaction of $\beta$-lactoglobulin with lactose as occurs during intensive heating (Maillard reactions) and others by brief pepsin hydrolysis of some of the milk proteins. These interesting possibilities have not been established as being responsible for "naturally occurring" milk allergies.

Some cases of cow's milk allergy are relieved by substituting goat's milk, but most are not. Cow's and goat's $\alpha$-lactalbumins are very similar antigenically, at least in in vitro tests, and so are their $\beta$-lactoglobulins. It would not be expected that either of these would be involved in cases relieved by use of goat's milk. The principal milk protein that differs between the two species is $\alpha_{s1}$-casein. It is prominent in cow's milk but virtually absent from

goat's. Whether cases of cow's milk allergy relieved by goat's milk actually are caused by $\alpha_{s1}$-casein is not known.

Heat denaturation of $\alpha$-lactalbumin and $\beta$-lactoglobulin diminishes their antigenic potency, but positive reactions to both have been observed with commercial evaporated milk in experiments in vitro and with guinea pigs. Apparently the heat treatment received in manufacture does not eliminate completely the antigenicity of these proteins.

As shown in Table 19.3, a principal difference between cow's and human milk is the high content of lactoferrin and lysozyme in the latter. Both of these proteins have marked effects on certain microorganisms, lactoferrin by depriving them of Fe and lysozyme by attacking cell wall constituents. It seems possible that these proteins may serve to regulate the kind and activity of the flora of the digestive tract of human infants receiving human milk, and thus that the flora might differ greatly from that of infants receiving cow's milk.

## 19.6. VITAMINS

Vitamins are essential organic compounds that animals require in the diet because they cannot synthesize them. In most cases the lack of synthetic ability is absolute, but in a few synthesis occurs but is not rapid enough to meet requirements. Vitamins range in molecular weight from 176 (ascorbic acid) to 1355 (vitamin $B_{12}$); their structures are given in the appendix (Table A.16). The discussion in this chapter is limited to origin and levels of vitamins in milk, variations caused by biological and processing conditions, and adequacy of milk in supplying human requirements. The specific biological functions of vitamins are not discussed.

Cow's and human milks contain all of the known vitamins, except that vitamin K is scarcely detectable in many specimens. Milk is the major source of some of the vitamins for human adults and infants. Tables 19.1 and A.14 (appendix) give the vitamin contents of milks of both species and Recommended Dietary Allowances. Vitamins A, D, E, and K occur in the fat globules and the others in the plasma of milk.

**Vitamin A.** Vitamin A (retinol) is a primary alcohol derivative of a polymer consisting of four isoprene units. It is formed by cleavage of provitamin A carotenoids, polymers of eight isoprenes. Of these, $\beta$-carotene has the highest vitamin A potency because it consists of identical halves and thus yields two retinol molecules; other carotenoids yield but one. Plants synthesize carotenoids but not retinol; animals cannot synthesize carotenoids but can split them to retinol. They secure their vitamin A requirements by ingestion of carotenoids in foodstuffs from plants and both carotenoids and retinol in those from animals. Until recently vitamin A activity was expressed in international units (IU), one IU being equivalent to 0.3 $\mu$g of retinol or

0.6 μg of β-carotene. The lower potency of β-carotene was based on experiments with rats showing that its enzymatic conversion to retinol was only about 50% efficient. The vitamin A potency of carotenoids is depressed by another factor—inefficiency of absorption in the intestine. For humans, about one-third of the carotenoids ingested is absorbed. (Retinol is assumed to be absorbed completely.) The overall utilization of β-carotene is taken, therefore, as one-sixth and other carotenoids as one-twelfth that of retinol. Current usage is to express vitamin A potency as retinol equivalents (RE):

$$\begin{aligned} 1\ \text{RE} &= 1\ \mu\text{g retinol} \\ &= 6\ \mu\text{g } \beta\text{-carotene} \\ &= 12\ \mu\text{g other provitamin A carotenoids} \\ &= 3.33\ \text{IU from retinol} \\ &= 10.00\ \text{IU from } \beta\text{-carotene.} \end{aligned}$$

Vitamin A is present in cow's milk as retinol, retinol esters, and carotenes. The content depends strongly on the amount of carotenoids in the feed. The ratio of retinol to carotene in cow's milk varies with breed. Jerseys and Guernseys, famous for their intensely yellow milk fat, convert less of the carotene to retinol than do other breeds. Some specimens of human milk fat have been reported to contain only retinol, others both retinol and β-carotene. In some countries beverage milk and skim milk often are fortified with vitamin A to about 200–500 RE·liter$^{-1}$. Vitamin A is stable to heat.

**Vitamin D.** Vitamin D includes a number of compounds with antirachitic activity. Ergocalciferol ($D_2$) occurs in plant material and can be formed by UV irradiation of ergosterol. Cholecalciferol ($D_3$) is formed by UV irradiation of 7-dehydrocholesterol in animals, particularly in the skin. Both $D_2$ and $D_3$ occur in milk coming from the feed and the irradiated skin of the cow, respectively. Potency is sometimes expressed in International Units; 1 IU = 25 ng cholecalciferol. The content of vitamins D in cow's milk is very variable but always low (5–50 IU·liter$^{-1}$). The vitamin D potency of human milk is also low (about 25 IU·liter$^{-1}$). Vitamin D potency of milk can be increased by (1) irradiating the cow, (2) feeding higher levels of irradiated plant material, (3) irradiating milk, or (4) adding concentrates to milk. The last of these methods is much used in some countries to fortify beverage milk to 400 IU·liter$^{-1}$ (equivalent to 10 μg cholecalciferol). Vitamins D are stable to heating of the milk.

Reports in the 1960s indicated apparent high levels of vitamin D sulfate in cow's and human milk on the basis of colorimetric tests. Assuming that vitamin D sulfate has the same antirachitic potency as vitamin D itself, these analyses indicated 950 and 200 IU·liter$^{-1}$ in human and bovine milks, respectively. Later analyses, particularly with high-performance liquid chromatography and ligand-binding analyses, discount the claim of high vitamin D sulfate contents. In fact, it could not be detected at all. Current opinion is that vitamin D occurs in milk partly as $D_2$ and $D_3$ and partly as derivatives hydroxylated at the 1, 25, and 26 positions.

**Vitamin E.** Vitamin E consists of a group of tocopherols. The most potent of these and the principal one in milk is α-tocopherol. This is a strong reductant and serves as an antioxidant protecting lipids from oxidation. The vitamin E content of milk is rather low. It depends to some extent on feed; summer milk generally has a higher concentration than winter milk. It is stable to heat but may be partly destroyed by intensive illumination in the presence of $O_2$. The vitamin E content of human milk is about ten times that of cow's milk.

**Vitamin K.** Vitamin K is present only in traces in milk, if at all. Human needs for this vitamin are supplied from consumption of plant materials containing it and by microbial synthesis in the digestive tract.

**Vitamin $B_1$.** Vitamin $B_1$ (thiamin) occurs in milk largely in the free state but a portion (18–45%) is phosphorylated, and a small fraction is bound to protein. The active biological form of thiamin is the pyrophosphate form; it acts as coenzyme to carboxylase. Apparently, no thiamin in this form is present in milk; the phosphorylated portion probably is the monophosphate. The thiamin content of milk is affected little by the feed of the cow or by season and breed. It diminishes rapidly during the first ten days postpartum and then more slowly to a relatively constant level at thirty to sixty days. The proportion in the phosphorylated form is greater in early lactation milk. Thiamin in milk is destroyed to the extent of only 3% or 4% by low pasteurization, but sterilization may destroy 20–45% of it. It is destroyed also by exposure to light or $O_2$. Human milk contains only about a third as much thiamin as cow's milk.

**Vitamin $B_2$.** Vitamin $B_2$ (riboflavin) occurs in milk mostly in the free form (65–95%), most of the remainder being in the form of the coenzyme flavin adenine dinucleotide (FAD). Probably some is in the form of riboflavin 5'-phosphate (sometimes called flavin mononucleotide, FMN) as well. Usually, but not always, higher contents of riboflavin are reported in milk in the spring and early summer when pasturing starts. Jersey and Guernsey milk contains 25–50% more riboflavin than milk from Holsteins or Ayrshires. The concentration in colostrum is two to four times that in later milk. Riboflavin is stable to heat treatment but is readily cleaved to lumichrome by exposure to light. It is the principal light-sensitive constituent of milk; its involvement in photooxidative processes is described in Section 11.5. The riboflavin concentration in human milk is about a fourth that in cow's milk.

**Niacin.** Niacin (nicotinic acid) and its amide, niacinamide (nicotinamide), constitute a vitamin that functions in the form of nucleotides ($NAD^+$ and $NADP^+$) as coenzymes for many dehydrogenases. The vitamin occurs in milk primarily as niacinamide at a concentration of 0.3–2.0 (mean 0.9) $mg \cdot liter^{-1}$ in cow's milk and about 1.5 $mg \cdot liter^{-1}$ in human milk. Differences

in content attributable to feed, breed, season, or stage of lactation are neither large nor very consistent The vitamin is stable to heat treatments used in dairy processes and to light

Many animals, including humans, can synthesize niacin from tryptophan Approximately 60 mg of dietary tryptophan is equivalent to 1 mg of niacin Milk is rather deficient in niacin per se, but the tryptophan content of its proteins makes it overall a good source of niacin equivalents (NE) In computing NE of milk in relation to human requirements, as in Table 19 1, a sixtieth of the tryptophan content, minus the amount needed to meet the actual tryptophan requirement, is added to the niacin content

**Pantothenic Acid.** Pantothenic acid is a dipeptide of 2,4 dihydroxy-3,3 dimethyl-butanoic acid linked by an amide linkage to 3-amino-propanoic acid It constitutes a part of coenzyme A, the business end of that molecule consisting of 2-aminoethanethiol linked as an amide to the carboxyl of the pantothenic acid Animals can assemble coenzyme A but lack the capacity to synthesize its pantothenate moiety Pantothenic acid occurs partly free and partly bound (perhaps as coenzyme A) in milk, but data are conflicting on the partition Its content in milk depends on feed, breed, season, or stage of lactation It is stable to heat and light encountered in processing milk The concentration of pantothenic acid in human milk is one-half to two-thirds that in cow's milk

**Vitamin $B_6$.** Vitamin $B_6$ consists of three active components—pyridoxin, pyridoxal, and pyridoxamine—all having a pyridine ring with a hydroxy-methyl group at position 5, and alcohol, aldehyde, and amine, respectively, at position 4 It is present in milk mostly as pyridoxal (70–95%), the rest being in the form of pyridoxamine Pyridoxine has not been detected in milk Pyridoxal phosphate may occur in milk, but methods of extraction would have hydrolyzed it The $B_6$ content of milk is somewhat higher when cows are on early pasture and in colostrum, but breed differences have not been observed It is stable to low pasteurization but partly destroyed by sterilization or exposure to light There is only about a sixth as much vitamin $B_6$ in human milk as in cow's milk

**Biotin.** Biotin is present solely in the free state in milk Biotin sulfoxide, a biologically inactive oxidation product, has been isolated from milk but may have been formed in the isolation process The biotin concentration of milk is relatively constant, being little affected by variations in feed, breed, season, stage of lactation, or processing Human milk contains about a fourth as much biotin as cow's milk

**Folic Acid.** Folic acid occurs in milk primarily in the form of 5-methyltetrahydrofolate Milk contains a specific protein, folate-binding protein, which specifically and strongly binds folate In cow's milk this protein is

present in excess of the folate; an additional 50 $\mu g \cdot liter^{-1}$ of the latter could be bound. Problems in analysis of milk for folate resulted in many reports of extremely low levels. The best current methods indicate that the concentration in cow's and human milk averages about 50 $\mu g \cdot liter^{-1}$. Goat's milk, on the other hand, has a markedly lower content of only about 6 $\mu g \cdot liter^{-1}$ although it has a large excess of the folate-binding protein. This vitamin is susceptible to loss by heating and exposure to $O_2$. It is protected by ascorbate, and its loss parallels that of the latter. Reports of natural variation in folate content, mostly based on older, less reliable methods, revealed little variation.

**Vitamin $B_{12}$.** Vitamin $B_{12}$ (cobalamin) is the only metal-containing vitamin. It contains an atom of Co, and it is the only known compound in which Co functions biologically. The content of $B_{12}$ in milk depends somewhat on the cobalt intake of the cow. In milk $B_{12}$ is in the form of cobalamin (not cyanocobalamin) and more than 95% of it is bound to protein. The sole or at least predominant source of $B_{12}$ for the cow, and hence for the milk, is biosynthesis by rumen microorganisms. The content does not vary greatly with feed, breed, season, or stage of lactation, except for a high content in colostrum. $B_{12}$ is fairly stable to pasteurization and stable to exposure to light. It is destroyed partly by sterilization. Human milk contains only about 5% as much $B_{12}$ as cow's milk.

**Vitamin C.** Vitamin C (L-ascorbic acid) is of little importance as a vitamin in cow's milk because of its low concentration in relation to human requirements and its lability to heat and oxidation. The source of L-ascorbic acid in milk is the feed of the cow and biosynthesis in liver. L-ascorbic acid is important in oxidation–reduction phenomena. (See Sections 11.4 and 11.5.) Oxidation of ascorbate to dehydroascorbate is reversible; hence, the total vitamin C potency of milk is the sum of the two. Further oxidation irreversibly cleaves the lactone ring and produces 2,3-diketogulonic acid, which is biologically inactive. As milk is secreted, only L-ascorbate is present, but oxidation soon produces L-dehydroascorbate. The oxidation is promoted by light by means of its influence on riboflavin. (See Section 11.5.) Pasteurization may cause a loss of 20% in vitamin C activity; sterilization may destroy it completely. Human milk contains two to two and a half times as much ascorbic acid as does cow's milk. A nursing infant consuming a liter of human milk a day would get about 40 mg ascorbic acid, which would probably satisfy its requirements.

**Other Nutrients.** Other nutrients known to be essential to other animals, but for which no dietary requirements are known, are choline, carnitine, *myo*-inositol, para-aminobenzoic acid, thioctic acid, and taurine. All of these are present in milk. Contents of choline, *myo*-inositol, and para-aminobenzoic acid are given in Table A.14 and carnitine in Table A.9 (appendix).

Thioctic acid is mentioned in Section 8 1 4 Taurine is necessary for proper development of nervous tissue and clearly is a dietary essential in some species (e g , cat) Bovine milk contains only 1–7 mg kg $^{-1}$ of taurine, human milk 40–50 mg kg$^{-1}$ There is evidence that formulas based on bovine milk may not supply sufficient taurine for human (especially preterm) infants

## 19.7. MILK PRODUCTS

The manufacture of milk products influences nutritive value by separation, dilution, or concentration of the components of milk, by the addition of nonmilk ingredients, and by some effects of processing treatments The effects of fractionating processes can be inferred from the structure and composition of milk, as exemplified in Table 1 3 (Further details on lipids are in Tables 5 1, 5 2, and 5 5, and on amino acids in the appendix, Table A 6) Table A 15 in the appendix presents a compilation on the composition of many milk products, including important nutrients Such data are ap proximate and may in some cases give little more than an order of magnitude because of natural variation in milk composition differences in formulation (e g , according to legal requirements, local customs cost price, or marketing considerations), and differences in processing conditions

The main processing operation that may cause loss of nutrients is heat treatment, which is discussed in Chapter 10 To be sure, mild heat treatments like low pasteurization or even UHT heating cause very little change in nutritive value The proteins can be affected in that serum proteins are denatured (which has no effect on nutritional quality), and casein loses its propensity to clot in the stomach (which loss may even be advantageous, at least to infants ) There is some loss of available lysine (Figure 10 4 and Table 19 5) from Maillard reactions (Section 10 4), but this has little effect on the nutritional quality because milk protein is comparatively rich in lysine, the same holds for Maillard reactions during long storage of sterilized products Very intensive heating causes some loss of digestibility and biological value presumably because of cross linking reactions as described in Section 10 3 Though it has been considered possible that some of the reaction products may be toxic to humans, no such toxicity has ever been ascertained, at least for heat treatments as occur during the manufacture of milk products

Some vitamins can be destroyed partly by heating as discussed in the preceding section Results are summarized in Table 19 5 Experimental data on such losses are widely variable The variation is partly the result of the conditions during heating being different, especially $O_2$ content as many of the reactions involved are oxidations Also the presence of catalysts, like Cu for vitamin C oxidation, affect the result Changes during storage show similar variations The oxidation of ascorbic acid plays a key role Vitamin C itself is more heat labile in the dehydroascorbic acid form Folic acid is

**Table 19.5. Approximate Loss (%) of Some Nutrients in Milk by Heat Treatments**

| | Pasteurization[a] | Boiling | UHT Heating | Sterilization[b] |
|---|---|---|---|---|
| Available lysine | <1 | ~5 | < 1 | 2–10 |
| Vitamin $B_1$ | ~5 | 5–10 | 5–10 | 20–50 |
| Vitamin $B_6$ | ~0 | <5 | <10 | 5–20 |
| Folic acid | <5 | ~5 | <20 | <30 |
| Vitamin $B_{12}$ | 0–10 | 5–20 | 5–20 | 20–80 |
| Vitamin C | 5–25 | 5–50 | 5–30 | >50 |

[a]For instance, 15 s at 74°C.
[b]Sterilization in bottles or cans.

protected by vitamin C. Oxidation products of vitamin C (perhaps diketogulonic acid) seem to attack vitamin $B_{12}$. Keeping the $O_2$ level low, particularly during heating but also during long storage ($O_2$ may, for instance, penetrate in UHT milk in cartons), minimizes loss of vitamins C, $B_1$, $B_{12}$, and folic acid. Protecting milk from exposure to light prevents loss of vitamin $B_2$.

Dried milk products can be stored for a long time without much loss of nutrients. Section 17.2.2 gives particulars about reaction rates in relation to water content. There may be loss of the fat-soluble vitamins A and E because of oxidation. Maillard reactions may proceed to a considerable extent if the powder is allowed to take up water.

Milk products ("formulas") can be prepared for specific dietetic purposes. Low-fat products provide a simple example. Another one is milk treated with lactase to make it better suited for people intolerant of lactose. (See Section 19.2.) In the preceeding sections many examples of differences between human and cow's milk are given, and such observations have led to the development of *humanized milk* or *infant formulas* for babies. A long-known and simple way to make the gross composition of cow's milk more similar to human milk is by diluting with water and adding sucrose; boiling the milk (which was done anyway for hygienic purposes) improves the clotting characteristics but reduces vitamin C potency. Better results can be obtained by making mixtures of (cow's) milk, cream, sweet whey, whey protein concentrates (discussed in Section 17.7), lactose, and water. This takes care of gross composition, including mineral composition, and protein quality (ratio of casein to whey proteins and amino acid pattern); clotting characteristics, buffer capacity, and renal solute load also are similar to those of human milk. Furthermore, part of the milk fat may be replaced by a vegetable oil (like corn oil) to provide more essential fatty acids and vitamin E, and the product may be fortified with vitamin C (to make up for loss caused by [illegible]ig) and possibly vitamin D and Fe.

**Fermented Milks.** Fermented milks of numerous types and names exist Most result from the action of lactic acid bacteria on milk (e g , sour milk, taette, cultured yogurt) or concentrated milk (e g , yogurt and dahi), buttermilk derives from the churning of cultured cream Some fermented milks contain other microorganisms kefir and kumiss (the latter made of mare's milk) contain yeasts producing alcohol, and viili contains some molds Fermented milks often are claimed to be particularly healthful In some conditions, fermented milks may be preferable because they do not readily propagate infectious diseases, the low pH prevents growth or causes killing of pathogenic microogranisms It often has been suggested that bacteria fermenting the milk may be implanted in the large intestine after ingesting the fermented milk A milk product (acidophilus milk) has even been designed that contains lactic acid bacteria usually present in the gut But there is no clear evidence that the consumption of such fermented milks has any specific effects The low pH of fermented milks may be advantageous, particularly to infants, as it diminishes the buffer action in the stomach, probably absorption of Ca is somewhat enhanced Fermented milks have a lowered lactose content, and this is advantageous to lactose intolerant consumers Fermentation may affect the content of nutrients, notably B vitamins, some may increase (e g , niacin), others decrease (e g , vitamin $B_{12}$), but the changes are rather variable and not very well studied Changes in energy content or essential amino acids because of fermentation are negligible

There is some concern about the stereo isomer of lactic acid present in fermented milks Mammals all have L(+) lactate as a metabolite, and it can be removed efficiently from the body, particularly by conversion into glucose (Cori cycle) D(−) lactic acid is not metabolized so readily, and it may accumulate in the blood, in extreme cases causing acidosis The World Health Organization recommends the daily intake of D(−) lactate to be less than 100 mg per kilogram of body weight In several fermented milks (e g , yogurts, acidophilus milk) part of the lactic acid is in the D(−) form, but excessive quantities must be consumed to reach the critical intake For young infants, however, the daily intake may become too high if certain fermented milks are fed exclusively

## *Suggested Literature*

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*Cultured Dairy Foods in Human Nutrition*, International Dairy Federation Bulletin, Document 159, 1983.

# 20

# Analysis of Composition

In this book we do not treat methods used to investigate the properties of milk or its constituents That is a vast subject, warranting separate treatment Analytical methods have become highly sophisticated, and an analyst often specializes in a certain analytical technique rather than in the analysis of a certain material But in any event, he or she must know the system being analyzed This book is meant to provide such knowledge, and the present chapter gives additional information for those interested in testing milk Moreover, some features of the classical methods for estimating composition are given, mainly to show what is really determined and what the limitations are

## 20.1. GENERAL ASPECTS

Reliable and valid analysis may pose many problems First is whether the variable determined is relevant to the question asked For instance, is a leukocyte count of the milk a good criterion for the incidence of mastitis? Potential butter yield of a lot of milk can be predicted accurately from its fat content, but cheese yield not so well from N content (casein content would be better) General rules about the relevance of a method cannot be given, each case must be decided on its own merits Some general considerations of other aspects are given below, they concern sampling, accuracy, and recalculation

### 20.1.1. Sampling

A representative sample is prerequisite to obtain reliable results For liquid milk products this usually is not difficult, as they can be made homogeneous

easily. A problem may be creaming, especially if enhanced by cold agglutination of the fat globules. The flocculated globules usually are redispersed by warming the milk to 40–45°C and then mixing thoroughly and cooling to room temperature. This procedure always is applied to cold stored samples, and it serves an additional purpose. If milk is kept at temperatures below 35°C part of the fat may crystallize, depending on temperature and duration. Solid fat has a higher density than liquid, and this increases the density of the milk by up to 0.15%, and more in cream. Warming up causes the fat to melt, and after cooling to room temperature it takes considerable time before fat crystallization becomes appreciable.

Obtaining a representative and homogeneous sample is less easy if the milk contains churned fat (butter granules or fat lenses), clustered fat globules (causing rapid creaming), or sedimenting particles (e.g., lactose crystals in sweetened condensed milk). Products like butter, cheese, and milk powder are more difficult to sample, and specialized methods have to be used.

A sample may change during storage. It may undergo physical (e.g., creaming or coalescence of fat globules), chemical (e.g., oxidative or photochemical reactions), enzymatic (e.g., lipolysis and proteolysis), and numerous microbial changes. The latter deterioration is often most prominent. Samples usually are kept under refrigeration to slow down microbial and enzymatic changes. But cold storage itself has effects, for instance, on salt equilibria and on casein micelles; some changes are irreversible, such as loss of substances from the fat globule membrane. A preservative may be added to prevent or diminish microbial growth; it often does not prevent enzyme action, and it may even initiate chemical changes, particularly in the proteins; we may need to correct for the change in composition caused by the mere addition (often including a solvent or a solid carrier). The sample may be churned partly if air is beaten in during transport.

### 20.1.2. Accuracy

To what extent do the results of an analytical determination deviate from the true or real value? Often, we do not know the latter, even if it has been defined precisely. We usually make shift with a standard or reference method, the outcome of which is accepted arbitrarily as the true result. When we apply a method to a number of different samples, its results ($u$) usually will deviate from those of the reference method ($x$). The deviations may be of different types (see also Figure 20.1):

1. Systematic deviations (usually called accuracy of the mean, though in fact it is the inverse of accuracy). The average value of $u$ found for all samples with a particular value of $x$ differs from $x$. The deviation should be estimated over the whole range of $x$, and this usually is done by standard regression analysis (if necessary, curvilinear regression). The deviation may be constant (blank), proportional to $x$ (wrong

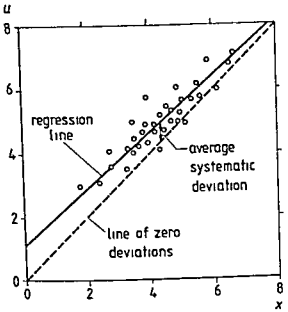



**Figure 20.1.** Hypothetical example of the results of determinations (say, of percentage fat in milk) on a number of samples by two different methods (results $u$ and $x$).

slope), both, or more complicated. Systematic deviations can be eliminated (if known); they have a sign and can be added to one another.

2. Stochastic deviations (usually called precision). After systematic deviation has been allowed for, random differences between $u$ and $x$ remain. Because of their stochastic nature (they often have a standard–normal distribution) they are characterized and expressed as a standard deviation. The squares of standard deviations (called variances) may be added to one another; hence the elimination of a comparatively small source of stochastic deviation does not materially alter the standard deviation (e.g., $\sqrt{4^2 - 1^2} \approx 3.9$).

Part of the cause of stochastic deviation is always the reproducibility (i.e., the deviations between results obtained by repeating the same method on the same sample). But this is usually not the main source of uncertainty. Milk is variable in composition and properties (which is the reason for analyzing it), and the variability itself is a cause for uncertainty in the result of the analysis. Rarely is the variable studied the only one that affects the outcome. Density and viscosity of the milk affect the mass of sample flowing out of a pipette. Some constituents vary in composition, and this may affect the result; for instance, fat composition affects its average molecular weight, and this causes variation in determination of weight fat percentage by a method that measures the number of moles. Other constituents may affect the outcome; a good example is compositional analysis by infrared spectrometry, where a certain absorption wavelength is usually not completely specific for one component. If, for example, the result of a method for determining component $x$ also is affected (somewhat) by component $y$, the outcome is $a[x] + b[y]$. The uncertainty in the determined $[x]$ is smaller if the selectivity $a/b$ is higher and if the variability in $[y]$ is less. The uncertainty also is affected by the correlation between $[x]$ and $[y]$. If this correlation is

high (either positive of negative), a simple correction in the proportionality constant $a$ improves the accuracy. An example is a method for fat content somewhat affected by protein content, as these values are usually correlated. Often, we know within certain limits what the average [y] is, for instance, because it varies with season; consequently, a correction for a blank ($= -b[\bar{y}]$) may be added.

The variability in composition and properties, and often the accuracy of an analytical method, depends on the sample population. Milk samples from individual cows usually show a wider range of variation than samples of mixed milk, and this holds for the quantity to be measured as well as for the disturbing factors. We usually are most interested in the ratio between the inaccuracy of a method and the range of the variable being estimated; if both are expressed as standard deviations, the ratio is $(1 - r^2)^{\frac{1}{2}}$, where $r$ is the correlation coefficient between the estimate and the true value.

Variability in composition may be particularly cumbersome if an analytical method is used to detect adulteration. For instance, addition of water to milk can be detected by the increase in freezing point caused by the added water. But the natural range in freezing point corresponds to a change caused by adding several percentages of water, detracting from the sensitivity of the method. Similar but larger problems arise in the detection of nonmilk fat in milk products by determining the proportion of butyric acid residues in the fat, or in the detection of dried whey in dried milk from the content of low molecular weight peptides.

### 20.1.3. Recalculation

If the content of a certain constituent is known in milk itself, we may desire to calculate the content in certain physical fractions of the milk or in products derived from it. The distribution of the component among the fractions (for many it is given in Table 1.3) and the manner and efficiency of separation applied in making the products must be known. Possible adsorption or exclusion of substances have to be taken into account. An important example is recalculation of the concentrations of dissolved substances (assuming that no dissolution or precipitation occurs). Some results are given in Table 20.1. It proceeds from the concentration $x$ of the dissolved substance (in moles, kilograms, or any other unit) per kilogram of milk. The factor 1.01 is needed to recalculate the content of fat to the content of fat globules; the factor 1.08 (in fact variable 1.06 – 1.10) to recalculate from casein to dry matter in casein micelles. The factor $h$ gives the amount of water (in grams of water per grams of protein) that is not available as solvent; it varies with molecular weight of the solute. Rough estimates are:

small molecules, e.g., $CO_2$, urea $h \approx 0.2$
lactose $h \approx 0.55$

**Table 20.1. Recalculation of the Concentration of a Milk Component to That in Plasma and Serum and Relative to Water**

| Product | Concentration per kg | Concentration per $m^3$ |
|---|---|---|
| Milk | $x$ | $x\rho_m$ |
| Plasma | $x/(1-1.01f)$ | $x\rho_p/(1-1.01f)$ |
| Serum | $x/(1-1.01f-1.08c-hc)$ | $x\rho_s/(1-1.01f-1.08c-hc)$ |
| Relative to water | $x/(1-t-hp)$ | $x\rho_w/(1-t-hp)$ |

*Note* $\rho$ = density (kg $m^{-3}$) with subscripts m, p, s and w denoting milk, plasma serum, and water, respectively $f$ = fat, $t$ = total dry matter, $p$ = protein, and $c$ = casein content, all as mass fraction (kg $kg^{-1}$)

The factor for serum proteins with respect to casein has never been determined, we assume it to be about 1.5. For example, if the content of serum proteins is 6 g $kg^{-1}$ of milk, then it is for a milk sample of average composition about 6.9 kg $m^{-3}$ serum, if the lactose content is 46 g $kg^{-1}$ of milk, it is about 53.9 g $kg^{-1}$ of water

## 20.2. SOLIDS AND SOLIDS-NOT-FAT

The total solids or dry matter content of milk and milk products may be important because product specifications include minimal standards and because the yield of dried products depends on it. The same may hold for the solids-not-fat or nonfat dry matter content. Otherwise, the development of rapid and easy methods for determination of protein and lactose contents has reduced the significance of total solids as a criterion for milk composition.

The classical determination of total solids depends on evaporation by oven drying to constant weight. This poses some problems:

1. Dried milk is very hygroscopic, and the final fraction of water is difficult to remove. Best results are obtained by applying a fairly high temperature (e.g., 102°C), using predried air. Water of crystallization of lactose is particularly persistent, consequently, the water should evaporate at such a temperature (> 94°C) that the anhydrous $\beta$ lactose crystallizes rather than $\alpha$-lactose hydrate. (See Section 3.2.) This can be achieved by evaporating most of the water on a boiling water bath, before oven drying.
2. Milk contains volatile components other than water, such as $CO_2$, short-chain fatty acids, and $NH_3$. These also are removed by drying.
3. Chemical reactions may occur, for instance, Maillard reactions causing weight loss or lipid oxidation causing weight gain. These effects increase with temperature and drying time.

It is not known precisely how well the oven-drying residue represents the nonwater part of milk. It appears as if the total of fat, protein, lactose, and known other components (e.g., Table 1.1) is slightly higher (e.g., 0.05 percentage units) than the residue after drying. Because the absolute variation in the content of other components is small, total solids content can be estimated from the sum of fat, protein, lactose, and a constant factor with fair accuracy (coefficient of variation less than 1% for individual cow samples).

We may directly determine water content, for instance, by chemical means (titration according to Karl Fischer) or from infrared absorption. For liquid products such methods are usually not very accurate; for instance, a relative error of 1% in the water content of milk causes a 7% error in total solids.

Another way to arrive at the solids content ($S$) is by calculation from density ($\rho$) and fat content ($F$). The density of milk can be determined readily and with sufficient accuracy with a lactometer (i.e., a specially adapted and calibrated hydrometer), provided that the precautions mentioned in Section 20.1.1 have been taken. The underlying idea is that the densities of milk fat ($\sim$ 917 kg·m$^{-3}$ at 20°C), solids-not-fat ($\sim$ 1622), and water (998) all are approximately constant. These values lead to

$$S = 1.23\,F + \frac{260(\rho^{20} - 998)}{\rho^{20}} \qquad (20.1)$$

expressed in percentage by weight, where $\rho^{20}$ denotes density of the milk. The difficulty is, of course, that the density of solids-not-fat is by no means invariable, because of the variation in composition. For every set of samples the equation may be different, and it would be best to obtain such an equation by multilinear regression between $S$, $F$, and $G$ ($G = \rho^{20} - 1000$). For instance, a set of individual cows' samples in the Netherlands yielded

$$S = 1.17\,F + 0.25\,G + 0.95 \pm 0.25 \qquad (20.2)$$

where the last term represents the residual standard deviation (which was 0.15% for herd milk samples). Consequently, calculation of solids content is of limited accuracy. In principle, the equations also can be used for liquids derived from milk by adding or removing cream or water (hence for skim milk, cream, evaporated milk), albeit with a still lower accuracy. But they are not applicable to products of other composition (e.g., whey, ultrafiltered milk, sweetened condensed milk).

## 20.3. FAT CONTENT

No milk testing method has been studied more extensively than determination of fat content. But, then, at least some $10^9$ fat tests are performed yearly worldwide, and the results often have important financial consequences. The fat content of milk is taken commonly to include all of its lipids (see Section 5.1), including polar lipids. This definition is not com-

pletely unequivocal, for example, is free butyric acid a lipid? But the uncertainty is mostly very small

In the generally accepted operational definition, the fat content of fresh milk is obtained by application of the gravimetric method of Rose–Gottlieb This method is very reproducible (standard deviation < 0 01% fat) Its principle is extraction with ether and light petroleum of a weighed quantity of milk to which ammonia and ethanol have been added to render the lipids extractable, and weighing the extracted material after solvent evaporation and drying A considerable fraction (about two-thirds) of the free fatty acids is lost, because they are in the dissociated state at the prevailing high pH and as such much more soluble in water than in apolar solvents Also, some polar lipids are lost (about 10% of the phospholipids), presumably because they are present in lipoproteins On the other hand some nonlipid material (again, lipoprotein components) are extracted Increasing the ionic strength of the milk solution (e g , by adding 1% NaCl) or using more ethanol increases the lipoprotein yield In fresh milk, the Rose–Gottlieb method gives a result that is about 0 01% fat below the true content of lipids But in other products, for instance, those containing much free fatty acids, the recovery may be less

In other gravimetric methods (such as the Weibull and the Schmidt–Bondzynski–Ratzlaff methods) the sample is boiled with HCl to digest protein and fat globule membrane material, after cooling the mixture is extracted with ethanol, ether, and light petroleum (SBR), or filtered and washed before extraction of the filter with ether (Weibull) Here, the loss of free fatty acids is lower (about 10%, largely because of evaporation) and that of phospholipids higher (about a third, largely because of hydrolysis) Moreover, extractable nonlipid substances are formed, particularly by reactions of sugars (yielding, e g , levulinic acid), hence the result may be too high, though this depends much on the composition of the product examined

Numerous methods have been devised for practical large scale fat testing The most accurate of these yield a relative standard deviation of difference from Rose Gottlieb of about 1% for individual cow samples Widely used are volumetric methods that employ specially designed vessels with narrow graduated stems A volume of milk is digested, usually with concentrated sulfuric acid, dissolving all of the protein and fat globule membranes The fat is then collected in the graduated stem by centrifuging Most used are the Babcock and the Gerber methods The latter employs a special vessel, usually called a butyrometer, and some pentanol to facilitate coalescence of the denuded fat globules This makes the method somewhat more reliable and versatile but also more elaborate The volumetric methods are largely empirical and have to be calibrated against a gravimetric method Very finely divided fat is not completely recovered (as in homogenized milk) The result depends rather closely on operational conditions (strength of sulfuric acid, quantities of reagents, temperature and time of digestion, acceleration, and time of centrifugation)

Several physical methods have been devised to allow rapid and automatic testing. Among these is turbidimetry of diluted milk, in which the scattering by casein micelles has been eliminated by dissolving them. It is clear from Section 11.2 that the turbidity depends not only on fat content but also on fat globule size and on the refractive index of the fat. The latter depends on fat composition (refractive index increases with average chain length and average number of double bonds of the fatty acid residues) and thus on cow (breed of), feeding regime, and stage of lactation. This may cause considerable uncertainty, particularly where feeding regimes are highly variable. The effect of variation in fat globule size can be minimized by homogenizing the milk in a reproducible way and by careful selection of wavelength and optical arrangements in the photometer (large angle of acceptance). In this way, the turbidity can be made virtually independent of average globule diameter, but variation in the width of the globule size distribution still affects the outcome. Consequently, homogenizing conditions are critical and any pretreatment of the milk leading to changes in globule size distribution may lower the accuracy.

Fat content of milk (and protein and lactose contents as well) can be estimated directly in undiluted samples by infrared absorption photometry. Light scattering by the fat globules has to be eliminated, and this can be achieved by reducing them in size by homogenization; the ratio of globule size to wavelength is then sufficiently small to make scattering negligible compared to absorption of light. Two wavelengths can be used for fat determination. At 3.4 $\mu$m the C–H stretching vibrations in the $CH_2$ groups are the primary cause of absorption. The concentration of $CH_2$ groups varies little in milk fat (though much between various other fats), permitting accurate determination of fat content. But other milk components, notably lactose, also absorb at 3.4 $\mu$m. Consequently, a correction for lactose has to be applied, which can easily be done in the instrument. For products with a low fat content (e.g., skim milk), however, the correction is relatively so large as to make the fat determination inaccurate. At 5.7 $\mu$m ester bonds absorb. The concentration of ester bonds in milk fat varies considerably with composition (even more than refractive index). Consequently, the accuracy strongly depends on variation in fat composition. Moreover, the result depends on lipolysis and on the (variable) content of nonlipid esters.

## 20.4. PROTEINS

Protein in milk classically has been determined by Kjeldahl analysis for N. Digestion with sulfuric acid and catalyst(s) oxidizes C to $CO_2$ and H to $H_2O$, but leaves N as $NH_4^+$. After alkalinization of the digest, $NH_3$ is distilled into standard acid and determined by titration. Estimation of protein by means of N analyses has the advantage that N is a major constituent, comprising about a sixth of the mass of the protein, and that the N contents of

individual milk proteins are nearly the same, as shown in the appendix, Table A 6 Multiplication by 6 38 has been used widely to convert N content to protein This is based on an old determination of 15 67% N in milk proteins, but, as Table A 6 shows, the principal milk proteins have slightly higher N contents than this, based on composition derived from sequence analysis The mean factor for these proteins, representing more than 95% of the total milk protein, weighted by their proportions (Table 6 3) is 6 32

Protein contents often are reported as "crude protein," calculated as 6 38 × total N Such values are 4–8% higher than the true protein contents because they include N from nonprotein nitrogenous constituents (See Section 8 1 4 ) True protein can be calculated from difference between total and nonprotein N as explained later in this section

The Kjeldahl method is somewhat cumbersome and time consuming, simpler and faster methods have been sought for routine assays of large numbers of samples in order to price milk partly on protein content Some procedures determine $NH_3$ directly in the digest and thus avoid distillation and titration Colorimetric analyses of $NH_3$ with Nessler's reagent, an alkaline mercuric iodide, to form a yellow complex, or with alkaline phenolic solutions and hypochlorite to form blue green indophenol, give reasonably satisfactory results The indophenol method seems well adapted for rapid automated analysis of numerous samples, a newer version uses salicylic acid instead of phenol

The Kofranyi method for determining milk protein measures ammonia that can be steam distilled from alkaline solution Essentially, this determines amide N plus a little more Under the conditions specified, approximately 1 25 mol $NH_3$ is released per kilogram of protein The weighted mean amide content of the principal milk proteins listed in Table A 6 is about 1 mol $kg^{-1}$ As compared with Kjeldahl this method suffers the disadvantage that it deals with a component, amide N, which is a much smaller and more variable portion of protein than is total N The fact that the distillable $NH_3$ is greater than amide N implies that some other partial cleavages occur in the process and that the conditions for the assay must be controlled rigidly

Colorimetric methods not involving digestion of the protein include biuret and Folin tests The former is a reaction of the peptide bond with alkaline copper reagent It has the advantage that individual proteins yield nearly identical quantitative response The Folin method involves reduction of a phosphotungstic acid reagent by aromatic side chain groups, primarily tyrosine, in the protein The extent of the reaction varies greatly with the number and location of these groups in the protein, consequently, the method is not suitable for determining total protein content of a mixture of components varying in proportions and in tyrosine content and sequence

The formol titration measures protons released when formaldehyde reacts with proteins Essentially, it is a measure of lysyl side chain amino groups For milk it is conveniently determined by first neutralizing a sample to the phenolphthalein end point (about pH 8 3), adding formaldehyde, and reti

trating. Complications caused by release and uptake of protons by various forms of calcium phosphate during the titration can be eliminated by precipitating all of the Ca with added oxalate before titrating. The titratable protons released by the action of formaldehyde amount to about 0.57 mol·kg$^{-1}$ of crude protein (often quoted as the reciprocal called "formol factor," i.e., 1.75 kg of protein per mole of alkali consumed). Correction to a true protein basis by accounting for 5% nonprotein N makes the value 0.60 mol $H^+$ per kilogram of protein. This is exactly the mean lysine content of the proteins listed in Table A.6, weighted by their concentrations. (See Table 6.3.)

The binding of anionic dyes to cationic groups in proteins in acid solution has been used widely as a means of determining the protein content of milk. The two dyes most used are Amido Black and Orange G. A milk sample is added to a solution of the dye at pH near 3.0, and the precipitated protein–dye complex is removed by centrifugation. The decrease in absorbance of the dye solution is proportional to protein content. Individual proteins differ somewhat in capacity to bind these dyes. Consequently, the result depends somewhat on variations in proportions of the proteins.

A turbidimetric method for whey protein (Harland–Ashworth method) has been used considerably in the dry milk industry. Casein and denatured whey protein are precipitated by saturating milk with NaCl, the resulting filtrate is acidified, and whey protein is estimated from its turbidity. Proteose–peptone components are precipitated with the casein in saturated NaCl, hence the maximal whey protein content determined by this method does not include them. The amount of undenatured whey protein depends on the cumulative heat treatments the milk has received in processing. The accuracy of this method for determining heat treatment is somewhat diminished by variation in the whey protein content of the original milk (on the order of 4–6 g·kg$^{-1}$).

Aromatic side chains in proteins absorb ultraviolet light of $\lambda = 280$ nm, but a suitable method of analysis based on this property has not been developed. A method based on measurement of fluorescence, light of $\lambda =$ 340–350 nm, emitted when the sample is illuminated with light of $\lambda = 240$–290 nm, has been proposed and tested. It suffers from the necessity for high dilution and for avoidance of contamination. Furthermore, the slope of the standard curve is so low that small errors in measuring fluorescence are reflected in large errors in protein concentration.

Absorption of infrared radiation at $\lambda = 6.46$ $\mu$m by the peptide bonds is suitable for determining milk protein. Instruments have been developed in which fat, protein, and lactose can be determined successively on a sample by reading at specific wavelengths in the infrared. Correction for the small effect of fat on absorption at 6.46 $\mu$m can be made automatically by the instrument. Interference by lactose at 6.46 $\mu$m is slight, and milk contains little other material absorbing at this wavelength; ionized carboxylic acids absorb slightly. Individual proteins differ little in absorption at 6.46 $\mu$m.

The indophenol colorimetric, Kofranyi alkaline distillation, formol titra-

tion, dye binding, and IR absorption methods have been found comparable to the Kjeldahl method in precision and accuracy Generally, they are standardized by determination of the regression of the parameter measured on crude protein (total N × 6 38) as determined by Kjeldahl on a series of milk samples Results are then calculated as "total protein," which, as previously mentioned, is 4–8% higher than "true protein " The dye binding and infrared absorption methods have gained the greatest acceptance for automated analysis of large numbers of samples These methods do not or only partly determine nonprotein N Proteolysis causes the results of the Kofranyi and formol titration methods to increase somewhat, those of the dye binding and IR methods to decrease, and those of the Kjeldahl and indophenol methods to remain unaltered

Some individual milk proteins can be determined by quantitative immunological reactions Such methods require specific antisera produced in experimental animals against purified specimens of the proteins to be determined Several sensitive techniques for immunological assay are available, including gel diffusion and radioimmunological assays These methods have been used successfully for $\alpha$-lactalbumin, $\beta$-lactoglobulin, serum albumin, and the several immunoglobulins (IgGl, IgG2, IgA, and IgM) They have not been very successful for caseins, probably because of the difficulty of preparing antisera of sufficient specificity and potency Milk enzymes can be assayed by methods based on their specific enzymatic activity, in a few cases (e g , xanthine oxidase) immunological assays are also available $\alpha$ lactalbumin can be determined by making it the limiting factor in an assay of lactose synthetase (see Section 7 3 2), but such a test has not been developed for routine use

Various procedures are used to separate milk proteins into fractions or individual components that can then be analyzed separately Classical fractionations involve precipitation of casein at pH 4 6 and precipitation of all proteins with trichloroacetic acid at final concentration of 12% (w/v) To avoid the necessity for washing the precipitates quantitatively, N is determined in the filtrates, and concentrations of casein and whey protein are calculated as follows

TN = Total nitrogen
NCN = Noncasein N, N in filtrate from sample adjusted to pH 4 6, 40°C, 1 10 dilution
NPN = nonprotein N, N in filtrate from sample adjusted to 12% trichloroacetic acid, room temperature, 1 5 dilution
Casein = 6 38 (TN–NCN)
Whey protein = 6 38 (NCN–NPN)

Use of such a procedure essentially defines casein as the mixture of proteins that precipitates at pH 4 6, 40°C, 1 10 dilution All intact caseins and the $\gamma$-casein fragments of $\beta$ casein (Section 6 3 4) do so precipitate thus, but the proteose–peptone components, most of which are $\beta$ casein fragments, do

not. They can be estimated in the filtrate at pH 4.6 of a sample that has been heated to denature the whey proteins. Heat-denatured whey proteins precipitate with the casein at pH 4.6, leaving proteose–peptone and nonprotein N in solution. Some further fractionations of the pH 4.6 filtrate (from unheated milk) with $Na_2SO_4$ at various concentrations have been suggested in an attempt to determine β-lactoglobulin and immunoglobulins.

Individual caseins can be assayed by determination of C-terminal end groups. Another method, developed in the past few years, is ion-exchange chromatography on columns of DEAE-cellulose or hydroxyapatite. The caseins are separated and can be determined individually by Kjeldahl or biuret. Gel filtration has been used to separate α-lactalbumin, β-lactoglobulin, and serum albumin for quantitative determination. They separate readily because of their large difference in molecular weight (14,000, 36,500, and 66,000, respectively). The data for caseins, α-lactalbumin, β-lactoglobulin, and serum albumin, reported in Table 6.3, were obtained by ion-exchange and gel filtration separations.

Electrophoretic separations of milk proteins have been used for quantitation of individual components. Casein and whey proteins are first separated and then each is subjected to electrophoresis. Free boundary electrophoresis at pH near 8 resolves the whey protein mixture rather satisfactorily. Scans of refractive index change yield patterns of peaks whose areas are proportional to the protein concentrations. Quantitative distributions of the whey proteins by this method are similar to those obtained by gel filtration. Free boundary electrophoresis does not resolve $\alpha_{s1}$-, $\alpha_{s2}$-, and κ-caseins; hence it is of no value for a complete analysis. Electrophoresis in gels or other supports has been used widely for qualitatively examining the proteins in a mixture. Attempts have been made to use such procedures for quantitation, either by staining and scanning the protein bands or eluting sections of the support and analyzing each eluate for protein. Best results seem to have been obtained with cellulose acetate as support and a dye bound equally by all of the proteins. Electrophoresis of whey proteins on cellulose acetate has in some cases yielded quantitative distributions similar to those obtained by gel filtration. Urea at concentrations up to 7 *M* can be used in gel or cellulose acetate electrophoresis to resolve the $\alpha_{s1}$-, $\alpha_{s2}$-, and κ-caseins. A method like this may prove to quantitate the caseins satisfactorily; this has not been achieved to date, however.

## 20.5. CARBOHYDRATES

Methods for determining lactose involve, respectively, oxidation of the aldehyde group, reaction with phenolic compounds, polarimetry, infrared absorption, enzymatic assay, and chromatography.

The aldehyde of the glucose moiety of lactose is oxidized in alkaline solution by hypoiodite, Cu, picrate, or ferricyanide. Potassium hypoiodite,

KIO, formed by reaction of KI with chloramine-T (*p*-toluenesulfonchloramide), oxidizes the aldehyde stochiometrically to carboxyl at room temperature. The other oxidants require heating, and the extent of oxidation depends empirically on the time and temperature of heating. Some of the methods involve heating the milk sample directly with oxidant (e.g., Fehling's solution); others are applied to a deproteinized filtrate. Picric acid is especially suitable because it serves as the protein precipitant and, after alkalinization of the filtrate, as the oxidant. Reactions with oxidative reagents are quantitated either colorimetrically (e.g., absorbance at 490 nm of picramate formed from picrate) or titrimetrically (e.g., titration with standard $Na_2S_2O_3$ to measure excess oxidant). Oxidative methods, of course, will include reductants other than lactose, but in cow's milk the amount of such materials (monosaccharides, ascorbate, thiols) is so low relative to lactose as to be negligible.

Second, lactose may be determined by reaction with such phenolic compounds as phenol, anthrone, or orcinol in the presence of concentrated sulfuric acid. A yellow chromagen is formed. Milk can be diluted greatly for this reaction, and the protein and fat need not be removed. The reaction is not specific; a wide variety of sugars and their derivatives react. Oligosaccharides and protein-bound carbohydrates react because they are hydrolyzed to free sugars by the sulfuric acid. In cow's milk other reactants are negligible relative to lactose.

A third general way of determining lactose is by measuring the optical rotation of a deproteinized filtrate prepared from milk. A weighed sample of milk is made to volume with precipitant solution and rotation determined on the filtrate obtained. To calculate lactose concentration, allowance must be made for the volume of precipitate, but workers have not always agreed on the exact value. Correction for the volume of precipitate is much greater for the polarimetric method than for some of the oxidimetric methods because the dilution is smaller. Thus, for polarimetry 65 g milk may be diluted to 100 ml, but for the picrate method 1 ml milk is diluted to 100 ml.

The specific rotation ($[\alpha]$ = the rotation in degrees of arc per centimeter of path length of a hypothetical aqueous solution of 1 ml containing 1 g anhydrous lactose) depends on several factors. First, it depends on the ratio of $\alpha$- to $\beta$-lactose. To ensure that mutarotation equilibrium (Section 3.2.1) has been attained, the solution should be kept at pH $\approx$ 9 for 15 min. Because of changes in mutarotation, $[\alpha]$ depends on temperature; $[\alpha]_D^{20}$ decreases by 0.04 for every K increase in temperature. Concentration has a small effect on the specific rotation, but up to 5% lactose the effect is negligible. Suggestions have been made that the specific rotation for lactose in the deproteinized filtrate from milk may not be quite the same as the value for lactose in water, but milk also contains traces of other compounds with rotary power. Finally $[\alpha]$ strongly depends on wavelength: $[\alpha]_D^{20}$, that is, for the sodium D line ($\lambda$ = 589 nm), is +55.5; $[\alpha]^{20}$ = +65.0 for $\lambda$ = 546; +105.8 for $\lambda$ = 436; and +156.0 for $\lambda$ = 365 nm. Consequently, specific rotation now usually

is measured at short wavelengths instead of the original 589 nm, because the larger value of $[\alpha]$ permits greater precision.

A fourth general way of analyzing milk for lactose content is by measurement of absorption of infrared radiation at 9.6 $\mu$m. This absorption is caused by the multiple hydroxyl groups in the molecule. Neither protein nor fat absorbs sufficiently at 9.6 $\mu$m to influence the determination of lactose significantly.

Most carbohydrates, also sugars other than lactose, may be quantitated in milk by gas–liquid chromatography (GLC). GLC analyses are made conveniently on trimethyl silyl derivatives because they are volatile enough to be separated readily on the column. An internal standard, consisting of a compound not present in milk, such as ribitol, can be added to the sample in known concentration. Ratios of the area of peak produced by the standard with those of peaks representing milk components facilitate calculation of the concentrations of the latter. Trimethyl silyl derivatives of sugar anomers are separated by GLC. Thus, a pattern for cow's milk will have peaks for $\alpha$- and $\beta$-D-lactose, $\alpha$- and $\beta$-D-glucopyranose, $\alpha$- and $\beta$-D-galactopyranose, D-galactofuranose, and *myo*-inositol.

Lactose and some other carbohydrates, notably lactulose and epilactose formed upon heating of milk, can be quantitated by High Performance Liquid Chromatography (HPLC) using an internal standard. By the use of a suitable column (of the anion exchanger type) they can be determined without derivatization; $\alpha$- and $\beta$-anomers can be separated. A clear filtrate is needed, for example, obtained by ultrafiltration. Adding 50% acetonitrile to milk causes the protein to precipitate; the filtrate can be used directly for HPLC.

Enzymatic assays for glucose and galactose in milk are possible with commercially available D-glucose and D-galactose oxidases obtained from fungi. Concentrations of about 0.1 m*M* glucose and 0.2 m*M* galactose have been reported by use of such methods. For some reason not yet understood, concentrations three to eight times greater have been found by gas–liquid chromatographic techniques. No comparisons of the two methods on the same samples have been reported. Lactose can be enzymatically quantitated by first hydrolyzing it with $\beta$-galactosidase; a series of ensuing enzymic reactions on glucose leads to an equivalent quantity of NADPH, which is photometrically determined. A blank in which no $\beta$-galactosidase is added yields free glucose. This method and HPLC are both convenient and accurate and tend to replace the older methods for quantitative determination of lactose.

For some reason, not apparent to us, several methods for determining lactose in milk (including both the Cu reduction and the polarimetric methods of the Association of Official Analytical Chemists in the U.S.A.) specify calculations that yield the results as lactose monohydrate and are thus 5.26% (360/342) higher than the actual lactose content. Publications in the literature have not always specified clearly whether lactose contents are reported on the anhydrous or hydrated basis.

## 20.6. SALT CONSTITUENTS

Because ash is determined simply and conveniently, it often is reported in gross analyses as a constituent of milk. It is, of course, the residue left after incineration. It differs in composition from milk salts because the following changes occur during incineration:

1. Organic salts, such as citrate, are destroyed.
2. Sulfur of the proteins is oxidized and appears as sulfate in the ash.
3. Ester-bound phosphate of the caseins and phospholipids is converted to inorganic phosphate.
4. Losses of carbonate as $CO_2$ and gains from oxidation of organic compounds greatly change the carbonate content.
5. Some loss of sodium and potassium chlorides may occur by volatilization although this is small if the temperature is kept below 600°C, which can be achieved by increasing the temperature in steps (programmed ashing).
6. Some formation of metallic oxides occurs.

Ashing often is used as a preliminary treatment before determining individual salt constituents such as Ca, Mg, or phosphate. It usually is judged to be complete when no black specks of carbon can be seen. Wet digestion with sulfuric acid and hydrogen peroxide or with nitric and perchloric acids sometimes are used to destroy organic material before analysis for minerals. Such procedures run less risk of loss of salt constituents than does dry ashing, if no precipitation occurs. In some procedures ashing is dispensed with and minerals are determined by injecting unfractionated, but usually diluted, samples directly into a flame photometer. Defatted and deproteinized extracts, usually acid, are used to determine organic salts such as citrate; sometimes they are used for analyses of mineral constituents as well.

Partition of salt constituents between the dissolved and colloidal particles can be achieved by equilibrium dialysis or by pressure ultrafiltration. In the latter technique pressures must be limited to about 1 atm to avoid changing the composition of the dissolved phase by pushing water through the filter faster than the dissolved components (so-called sieving effect). Concentrations of elements in milk are best expressed in units of mass of the element per kilogram of milk (not, as formerly, in terms of their oxides).

General methods for quantitating minerals (especially metal elements) make use of absorption or emission of light of specific wavelengths. The former is a measure of the absorption of energy to raise electrons to a higher-energy excited state, and the latter measures the energy released when the excited electrons revert to their original state of energy. Emission instruments employ either electric arcs or flames to activate the sample. The atomic absorption and emission methods are particularly suitable for analyzing for

Na and K; for neither of these elements are suitable volumetric or gravimetric methods of sufficient sensitivity available. Also, the emission and absorption methods are by far the best for trace metal analysis because of their great sensitivity (0.1–10.0 ng with the so-called flameless methods) and because several metals can be determined successively on a sample by shifting the wavelength. Different metals may, however, require different methods of ashing and preparation. Ca and Mg also can be determined by flame photometry or atomic absorption but often have been analyzed by specific methods.

Ca was determined classically by precipitation as the oxalate which was then titrated in $H_2SO_4$ solution with $KMnO_4$. This method is somewhat tedious and has been replaced by two simpler ones. One is titration with the chelating agent ethylenediamine tetraacetate (EDTA), using as indicator a dye (murexide) that changes color when it binds Ca. The other is a colorimetric method using glyoxal bis(2-hydroxyanil), which forms a complex with Ca having an absorption maximum at 524 nm. The colorimetric method is considerably more sensitive than the EDTA titration. About 0.3 $\mu$g of Ca in 1 ml volume and 1 cm light path gives an $A_{524} = 0.1$. The EDTA solution generally is standardized to 1 mg Ca per milliliter; a titer of 0.1 ml would be equivalent to 100 $\mu$g. Phosphate interferes with both of these methods. It can be removed by passing the solution to be analyzed through an anion exchanger in the acetate form. Other ways of avoiding interference by phosphate are to precipitate it from the milk along with protein with potassium metastannate or to precipitate Ca as the oxalate before titrating with EDTA.

Mg formerly was determined by precipitation as magnesium ammonium phosphate and determining the phosphate content of the latter. At present it usually is determined by titration with EDTA. It can be obtained as the difference between two titrations, one with an indicator like murexide that measures Ca and the other with eriochrome black that detects both Ca and Mg. Another way is to titrate the supernatant left after precipitating Ca as the oxalate.

Phosphate is determined almost universally by use of its property of reacting with molybdate to form phosphomolybdate. Gravimetric methods based on isolating and weighing the phosphomolybdate have given way to colorimetric ones of great sensitivity based on reduction of the phosphomolybdate to a blue-colored compound. In one method 1 $\mu$g of P in 1 ml and 1 cm light path gives $A_{820} \approx 0.1$. Arsenate is the only serious interfering compound and is, of course, virtually absent from milk ($\leq 0.1$ $\mu g \cdot kg^{-1}$). Partition of phosphorus among the various classes of compounds in milk can be determined by making the following analyses:

I. Total phosphorus in a dry-ashed or wet-ashed sample.
II. Lipid phosphorus in a digested Röse–Gottlieb extract.
III. Dissolved phosphorus in a digested sample of ultrafiltrate.
IV. Inorganic dissolved phosphorus in an undigested sample of ultrafiltrate.

V Acid-soluble phosphorus in a filtrate made with 12 5% trichloroacetic acid

Then

$$\text{Lipid P} = \text{II}$$
$$\text{Casein P} = \text{I} - (\text{II} + \text{V})$$
$$\text{Inorganic dissolved P} = \text{IV}$$
$$\text{Inorganic colloidal P} = \text{V} - \text{IV}$$
$$\text{Water-soluble ester P} = \text{III} - \text{IV}$$

Chloride usually is determined by reaction with Ag to form an insoluble precipitate Direct titration of milk with silver nitrate and potassium chromate indicator yields results that are erroneously high and variable Preashing of milk runs the risk of loss of chloride by volatilization The best procedure seems to be to add directly to milk an excess of standardized silver nitrate, and titrate the excess with standardized potassium thiocyanate (KSCN) Ferric ammonium sulfate is used as indicator, the $Fe^{3+}$ ion forming a red salt with thiocyanate

Citrate may be oxidized with $KMnO_4$ and brominated and decarboxylated to form pentabromacetone Certain methods for determining citrate in milk, including that of the Association of Official Agricultural Chemists, U S A , are based upon forming, isolating, and weighing pentabromacetone Such methods are cumbersome and time consuming Furthermore, close control of the procedures is necessary because pentabromacetone is somewhat more soluble and volatile than desired for a compound to be determined gravimetrically Another method involves precipitation of lead citrate from a sulfuric acid alcohol filtrate of milk and oxidation of the lead citrate with ammonium perchlorato cerate This offers some advantages over the pentabromacetone method but is by no means as simple, sensitive and convenient as a colorimetric procedure in which the absorbance of a condensation product of citrate with pyridine in acetic anhydride is measured The reaction is conducted conveniently in a trichloroacetic acid filtrate of the milk As little as 5 $\mu$g of citrate can be determined by this method Enzymic assay of citrate is possible also, it involves the use of citrate lyase, malate dehydrogenase, and lactate dehydrogenase

## *Suggested Literature*

Standard methods of analysis for gross composition and some other compounds are given in

*Official and Tentative Methods of Analysis* 13th ed Association of Official Analytical Chemists 1980

and in the *Analytical IDF Standards* published by the International Dairy Federation, Brussels.

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D. T. Davies and J. C. D. White, *J. Dairy Res.* 29 (1962): 285.

For citrate by

J. C. D. White and D. T. Davies, *J. Dairy Res.* 30 (1963). 171.

And for lactose by

F. H. Grimbleby, *J. Dairy Res.* 23 (1956): 229.

Enzymic assays are described in

*Methods of Enzymatic Food Analysis*, (Mannheim. Boehringer, 1980)

# APPENDIX


| | | |
|---|---|---|
| Table A.1. | Trace Elements in Bovine and Human Milks | 396 |
| Table A.2. | Phosphate Esters in Milk | 398 |
| Table A.3. | Minor Fatty Acid Residues in Milk Fat | 398 |
| Table A.4. | Various Neutral Lipids in Milk Fat | 399 |
| Table A.5. | Milk Fat Constants | 401 |
| Table A.6. | Amino Acid Composition of Some Milk Proteins | 402 |
| Table A.7. | Amino Acid Sequences | 404 |
| Table A.8. | Location of Amino Acid Substitutions in Genetic Variants of Milk Proteins | 407 |
| Table A.9. | Various Nonprotein Nitrogenous Compounds in Milk | 408 |
| Table A.10. | Enzymes in Bovine Milk | 409 |
| Table A.11. | Alcohols, Carboxyls, Acids, and Esters in Milk | 412 |
| Table A.12. | Nucleic Acids and Nucleotides in Milk | 413 |
| Table A.13. | Miscellaneous Compounds Occurring as Conjugates in Milk | 414 |
| Table A.14. | Vitamins in Cow's and Human Milks | 415 |
| Table A.15. | Composition of Some Milk Products | 416 |
| Table A.16. | Formula and Properties of Milk Components | 423 |
| Table A.17. | Properties of Lactose | 450 |
| Table A.18. | Properties of Lactic Acid | 452 |
| Table A.19. | Intrinsic Solubility Products at 20°C | 452 |
| Table A.20. | Approximate Intrinsic Association Constants | 453 |
| Table A.21. | Physical Properties of Milk Fat | 454 |
| Table A.22. | Density and Viscosity of Milk Fractions as a Function of Temperature | 455 |
| Table A.23. | Refractive Indices of Milk Fractions | 456 |
| Table A.24. | Thermal Properties | 456 |
| Table A.25. | Nutritive Value of Milks | 457 |

**Table A.1. Trace Elements in Bovine and Human Milks ($\mu g \cdot kg^{-1}$)**

| Element | Symbol | Cow's Milk | | Human Milk | Requirement ($\mu g \cdot day^{-1}$) | |
|---|---|---|---|---|---|---|
| | | Contents Reported | Typical Value | | Children 1–2 yr | Adult |
| Aluminum | Al | 50–2,100 | 500 | 350 | ? | ? |
| Arsenic | As | 10–400 | 50? | 4 | ? | ? |
| Barium | Ba | tr–110 | tr | | +? | +? |
| Boron | B | 30–800 | 200 | 80 | 0 | 0 |
| Bromine | Br | 60–25,000 | 600? | 90–3,800 | +? | +? |
| Cadmium | Cd | tr–160 | <0.5? | 15–20 | P | P |
| Cesium | Cs | 3–46 | ? | | | |
| Chromium | Cr | 5–82 | 15 | 40 | 20–80 | 50–200 |
| Cobalt | Co | 0–20 | 1 | 0.02–2 | + | + |
| Copper | Cu | 10–1,200 | 25 | 30–1,300 | 1,000–1,500 | 2,000–3,000 |
| Fluorine | F | 20–700 | 180 | | +? | +? |
| Iodine | I | 5–700 | 50 | 25–900 | 70 | 150 |
| Iron | Fe | 100–2,400 | 200 | 1,000 | 7,000–15,000 | 10,000–18,000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Lead | Pb | 1–500 | <5 | tr–280 | P | P |
| Lithium | Li | tr–29 | tr | | 0 | 0 |
| Manganese | Mn | 3–370 | 20 | 15–55 | 1,000–1,500 | 2,500–5,000 |
| Mercury | Hg | ≤0 1 | tr | 1–100? | P | P |
| Molybdenum | Mo | 5–150 | 70 | 2 | 50–100 | 150–500 |
| Nickel | Ni | 0–180 | 30? | 8–80 | ? | ? |
| Rubidium | Rb | 100–3,400 | 2,000? | 630 | 0 | 0 |
| Selenium | Se | 4–1,200 | 40? | 10–400 | 20–80 | 50–200 |
| Silicon | Si | 1,300–7,000 | 1,400 | 800–1,900 | ? | ? |
| Silver | Ag | tr–54 | ? | | P | P |
| Strontium | Sr | 5–2 000 | 170? | | +? | +? |
| Tin | Sn | 0–1,000 | tr | 4 | ? | ? |
| Titanium | Ti | 2–500 | ? | 5–30 | ? | ? |
| Vanadium | V | tr–310 | 0 1? | | | |
| Zinc | Zn | 220–19,000 | 3,900 | 140–4,000 | 10,000 | 15,000 |

*Note* P = poisonous, 0 = no physiological role ? = physiological role questionable, +? = probably has physiological role, + = indispensable but requirement not known

## Table A.2. Phosphate Esters in Milk

| Compound | mg·kg$^{-1}$ |
|---|---|
| Glycerol phosphate | 10.0 |
| Phosphopyruvate | 0.1 |
| Phosphoethanolamine | 83.0 |
| Phosphoglycerolethanolamine | 46.0 |
| Phosphoserine | 0.7 |
| Pentose phosphates | + |
| Glucose-1-phosphate | 1.0[b] |
| Glucose-6-phosphate | 12.0 |
| N-acetyl glucosamine-1-phosphate | 89.0 |
| Galactose-1-phosphate | 45.0 |
| Fructose-6-phosphate | 4.0[b] |
| Fructose-1,6-diphosphate | 15.0 |
| Lactose-1-phosphate | 0.1 |
| Lactose-3′-phosphate[a] | 15.0 |
| Lactose-6′-phosphate[a] | |

*Note:* See also Table A.12

[a]3′ and 6′ refer to positions on galactose moiety.

[b]Another author reported 291 mg Glc-1-P kg$^{-1}$ and 655 mg Fru-6-P kg$^{-1}$.

## Table A.3. Minor Fatty Acid Residues in Milk Fat

| Type | Number Known | $x$ | $\bar{x}$ | Amount (mole %) |
|---|---|---|---|---|
| Acetic acid | | 2 | | 0.5 |
| Saturated, $x$ even, $x > 18$ | 5 | 20–28 | 21.9 | 0.4 |
| Saturated, $x$ odd | 12 | 5–27 | 16.2 | 1.8 |
| Saturated, monobranched | 22 | 9–26 | 16.4 | 1.6 |
| Saturated, multibranched | 16 | 16–28 | 22.6 | 0.7 |
| Cyclopropenoic | > 1 | ? | ? | 0.001 |
| Monoenoic, $x < 19$, *cis*[a] | 36 | 10–18 | 15.8 | 3.8 |
| Monoenoic, $x < 19$, *trans* | 25 | 12–18 | 17.7 | 3.0 |
| Monoenoic, $x > 18$ | 14 | 19–26 | 20.4 | 0.3 |
| Dienoic, *cis cis* | 13 | 14–26 | 18.0 | 1.9 |
| Dienoic, *trans trans* | 4 | 18 | | 0.1? |
| Dienoic, *cis trans* | 4 | 18 | | 0.3 |

**Table A.3. (*Continued*)**

| Type | Number Known | $x$ | $\bar{x}$ | Amount (mole %) |
|---|---|---|---|---|
| Trienoic | 7 | 18–24 | 18 6 | 1 0 |
| Tetraenoic | 3 | 18–22 | 19 7 | 0 2[b] |
| Penta and hexa enoic | 4 | 20–22 | 21 2 | 0 1 |
| Ketoacids | 47 | 10–24 | ~ 18 0 | 0 3[c] |
| Hydroxyacids | 26 | 6–16 | ~ 15 0 | 0 3[d] |
| Fatty alcohols | ~ 10 | 14–20 | ? | 0 01 |
| Fatty aldehydes | ~ 10 | 9–18 | ? | 0 02 |

*Note* As far as known (i e , mostly ≥ 0 01 mole %) Approximate results $x$ = number of C atoms

[a]Excluding oleic acid (*cis* $C_{18:1}\Delta 9$)
[b]Of which arachidonic acid (all *cis* $C_{20:4}\Delta 5\ 8\ 11\ 14$) 0 12 mole %
[c]0 02% 3 ketoacids
[d]0 02% 4 and 5 hydroxy acids

**Table A.4. Various Neutral Lipids in Milk Fat**

| Component | mg kg$^{-1}$ Lipid |
|---|---|
| Neutral plasmalogens | 400 |
| Neutral glyceryl ethers | 200 |
| γ-lactones ($x$ = 10–12) | < 0 5 |
| δ-lactones ($x$ = 8, 10–12, 14, 16) | 10 |
| β-methyl ketones ($x$ = 5–11, 13) | 0-0 5 |
| Cholesterol | 3000 |
| Campesterol | 20 |
| Stigmasterol | 5 |
| β-sitosterol | 10 |
| Ergosterol | ? |
| $\Delta^5$ avenasterol | 1 ? |
| 7 dehydrocholesterol | + |
| 22 dehydrocholesterol | tr |
| Desmosterol | tr |
| 24 methylene cholestrol | + |
| Lathosterol | tr |
| Fucosterol | 3 |
| Lanosterol | 70 |
| Steroidal ketones[a] | + |
| Dihydrolanosterol | 5 |

Table A.4. (*Continued*)

| Component | mg·kg$^{-1}$ Lipid |
|---|---|
| Aliphatic alcohols (free)[b] | 100. ? |
| Aliphatic hydrocarbons[c] | 100. |
| PAH[d] | 0.001 |
| Phytenes | + |
| Squalene | 60. |
| Waxes | tr |
| Ubiquinone | 4. |
| S-containing lipids (lipoic acid ?) | + |
| Calciferol (vit. $D_3$) | 0.02 |
| Vitamin K (menaquinone derivatives) | 2. |
| Carotenoids[e] | 8. (0.2–30.) |
| Xanthophyll (lutein) | 4. |
| Retinol | 8. (4.–20.) |
| $\alpha$-tocopherol | 30. |
| $\beta$-, $\gamma$-, $\delta$-tocopherol | + |
| $\alpha$-, $\gamma$-tocotrienol | + |

*Note:* Approximate results from various sources

[a]Includes $\Delta^4$-cholesten-3-one, $\Delta^{3,5}$-cholestadiene-7-one, and $\Delta^7$-cholesten-3-one.

[b]$x$ = 18–31, $y$ = 0–1 ($x$ = number of C atoms; $y$ = number of double bonds) Includes phytol and dihydrophytol.

[c]$x$ = 14–34, $y$ = 0–1. Both the aliphatic alcohols and hydrocarbons consist of odd- and even-numbered saturated and mono-unsaturated, straight and branched chains. Includes pristane (~ 70) and phytane (~ 8 mg·kg$^{-1}$)

[d]Polycyclic aromatic hydrocarbons, e g , 3,4-benzopyrene

[e]Mostly $\beta$-carotene, but also $\alpha$-, $\gamma$-, $\zeta$-carotene, lycopene, cryptoxanthin, zeaxanthin, and neo-$\beta$-carotene

## Table A.5. Milk Fat Constants

**Saponification Value**

SV = mg KOH needed to saponify 1 g of fat. Average molecular weight of triglycerides equals 168,300/SV. SV $\approx$ 56,100/ (45 + 14 $\bar{x}$)[a]. SV milk fat: 220–240.

**Iodine Values**

IV = g of $I_2$ reacted with 100 g of fat. $\bar{y}^b$ = 6.63 IV/SV.IV milk fat: 26–42.

**Refractive Index**

$n$, mostly for light of 589 nm wavelength and 40°C. $n_D^{40}$ increases with $\bar{x}$ and $\bar{y}$; on average ~1.455, range mostly 1.452–1.458.

**Reichert–Meissl–Wollny Number**

RMW = ml 0.1 N alkali needed to neutralize the water-soluble fatty acids derived from the steam distillate of 5 g saponified fat. RM milk fat: 20–35.

**New Kirschner Number or Butyric Acid Number**

NK = ml 0.1 N alkali needed to neutralize the fatty acids soluble in a $AgNO_3$ solution, and derived from the steam distillate of 5 g saponified fat. NK milk fat : 18–30.

**Polenske Number**

PN = ml 0.1 N alkali needed to neutralize the water-insoluble fatty acids derived from the steam distillate of 5 g saponified fat. PN milk fat : 1.0–3.3.

**Acid Value**

AV = mg KOH needed to neutralize the free fatty acids in 1 g of fat. AV of fresh milk fat : ~0.3. Fat acidity is often expressed in mmol/100 g fat (= 1.78 times AV); or as percentage (w/w) of oleic acid (= 0.50 times AV).

[a] $\bar{x}$ = average number of C atoms per fatty acid residue
[b] $\bar{y}$ = average number of double bonds per fatty acid residue.

**Table A.6. Amino Acid Composition of some Milk Proteins (mol per kg protein)**

| Component | MW | Casein | $\alpha_{s1}$-Casein (B) | $\alpha_{s2}$-Casein | $\beta$-Casein ($A^2$) | $\kappa$-Casein (B) | $\alpha$-Lact albumin (B) | $\beta$-Lacto globulin (B) | Blood Serum Albumin |
|---|---|---|---|---|---|---|---|---|---|
| N | 14.1 | 11.30 | 11.25 | 11.34 | 11.27 | 11.62 | 11.60 | 11.25 | 11.74 |
| P | 31.0 | 0.26 | 0.34 | 0.44 | 0.21 | 0.05 | 0.00 | 0.00 | 0.00 |
| S | 32.1 | 0.23 | 0.21 | 0.24 | 0.25 | 0.22 | 0.63 | 0.49 | 0.59 |
| Glycine (Gly) | 75.1 | 0.25 | 0.38 | 0.08 | 0.21 | 0.11 | 0.42 | 0.22 | 0.23 |
| Alanine (Ala) | 89.1 | 0.36 | 0.38 | 0.32 | 0.21 | 0.79 | 0.21 | 0.82 | 0.71 |
| Valine (Val) | 117.2 | 0.62 | 0.47 | 0.55 | 0.79 | 0.58 | 0.42 | 0.49 | 0.54 |
| Leucine (Leu) | 131.2 | 0.74 | 0.72 | 0.52 | 0.92 | 0.42 | 0.92 | 1.20 | 0.94 |
| Isoleucine (Ile) | 131.2 | 0.47 | 0.47 | 0.44 | 0.42 | 0.68 | 0.56 | 0.55 | 0.21 |
| Proline (Pro) | 115.1 | 1.02 | 0.72 | 0.40 | 1.46 | 1.05 | 0.14 | 0.44 | 0.42 |
| Phenylalanine (Phe) | 165.2 | 0.33 | 0.34 | 0.24 | 0.38 | 0.21 | 0.28 | 0.22 | 0.39 |
| Tyrosine (Tyr) | 181.2 | 0.34 | 0.42 | 0.48 | 0.17 | 0.47 | 0.28 | 0.22 | 0.29 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Tryptophan (Trp) | 204.2 | 0.06 | 0.08 | 0.08 | 0.04 | 0.05 | 0.28 | 0.11 | 0.03 |
| Serine (Ser) | 105.1 | 0.68 | 0.68 | 0.67 | 0.67 | 0.68 | 0.49 | 0.38 | 0.42 |
| Threonine (Thr) | 119.1 | 0.38 | 0.21 | 0.59 | 0.38 | 0.74 | 0.49 | 0.44 | 0.51 |
| Cysteine (Cys) | 121.2 | 0.02 | 0.00 | 0.08 | 0.00 | 0.11 | 0.56 | 0.27 | 0.53 |
| Methionine (Met) | 149.2 | 0.21 | 0.21 | 0.16 | 0.25 | 0.11 | 0.07 | 0.22 | 0.06 |
| Arginine (Arg) | 174.2 | 0.22 | 0.25 | 0.24 | 0.17 | 0.26 | 0.07 | 0.16 | 0.35 |
| Histidine (His) | 155.2 | 0.19 | 0.21 | 0.12 | 0.21 | 0.16 | 0.21 | 0.11 | 0.26 |
| Lysine (Lys) | 146.2 | 0.56 | 0.59 | 0.95 | 0.46 | 0.47 | 0.85 | 0.82 | 0.91 |
| Asparagine (Asn) | 132.1 | 0.31 | 0.34 | 0.55 | 0.21 | 0.37 | 0.85 | 0.27 | 0.17 |
| Aspartic acid (Asp) | 133.1 | 0.22 | 0.30 | 0.16 | 0.17 | 0.21 | 0.63 | 0.55 | 0.63 |
| Glutamine (Gln) | 146.2 | 0.74 | 0.64 | 0.59 | 0.88 | 0.74 | 0.35 | 0.49 | 0.26 |
| Glutamic acid (Glu) | 147.1 | 0.87 | 1.02 | 0.99 | 0.75 | 0.68 | 0.56 | 0.88 | 0.94 |
| g protein per g N | | 6.318 | 6.346 | 6.296 | 6.335 | 6.144 | 6.155 | 6.346 | 6.081 |
| MW protein | | 23,192 | 23,615 | 25,230 | 23,983 | 19,025 | 14,176 | 18,283 | 66,267 |

Calculated from the amino acid sequence of each protein, including (organic) phosphate but not carbohydrate groups.

*β-Casein A*[2]

```
                                                                    10                  20
H Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser-Leu-Ser-Ser-Ser-Glu-
                                                                     |   |   |   |
                                                                     P   P   P   P
                                                                    30                  40
Glu-Ser-Ile-Thr-Arg-Ile-Asn-Lys-Lys-Ile-Glu-Lys-Phe-Gln-Ser-Glu-Glu-Gln-Gln-Gln-
                                                                     |
                                                                     P
                                                                    50                  60
Thr-Glu-Asp-Glu-Leu-Gln-Asp-Lys-Ile-His-Pro-Phe-Ala-Gln-Thr-Gln-Ser-Leu-Val-Tyr-

                                                                    70                  80
Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-Gln-Thr-

                                                                    90                  100
Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro-Glu-Val-Met-Gly-Val-Ser-Lys-Val-Lys-Glu-

                                                                    110                 120
Ala Met-Ala-Pro-Lys-His-Lys-Glu-Met-Pro-Phe-Pro-Lys-Tyr-Pro-Val-Gln-Pro-Phe-Thr-

                                                                    130                 140
Glu-Ser-Gln-Ser-Leu-Thr-Leu-Thr-Asp-Val-Glu-Asn-Leu-His-Leu-Pro-Pro-Leu-Leu-Leu-

                                                                    150                 160
Gln-Ser-Trp-Met-His-Gln-Pro-His-Gln-Pro-Leu-Pro-Pro-Thr-Val-Met-Phe-Pro-Pro-Gln-

                                                                    170                 180
Ser-Val-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Val-Pro-Glu-Lys-Ala-Val-Pro-Tyr-

                                                                    190                 200
Pro-Gln-Arg-Asp-Met-Pro-Ile-Gln-Ala-Phe-Leu-Leu-Tyr-Gln-Gln-Pro-Val-Leu-Gly-Pro-

                                                                209
Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val OH
```

*κ-Casein B*

```
                                                                    10                  20
PyroGlu-Glu-Gln-Asn-Gln-Glu-Gln-Pro-Ile-Arg-Cys-Glu-Lys-Asp-Glu-Arg-Phe-Phe-Ser-Asp-

                                                                    30                  40
Lys-Ile-Ala-Lys-Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg-Tyr-Pro-Ser-Tyr-Gly-Leu-

                                                                    50                  60
Asn-Tyr-Tyr-Gln-Gln-Lys-Pro-Val-Ala-Leu-Ile-Asn-Asn-Gln-Phe-Leu-Pro-Tyr-Pro-Tyr-

                                                                    70                  80
Tyr-Ala-Lys-Pro-Ala-Ala-Val-Arg-Ser-Pro-Ala-Gln-Ile-Leu-Gln-Trp-Gln-Val-Leu-Ser-

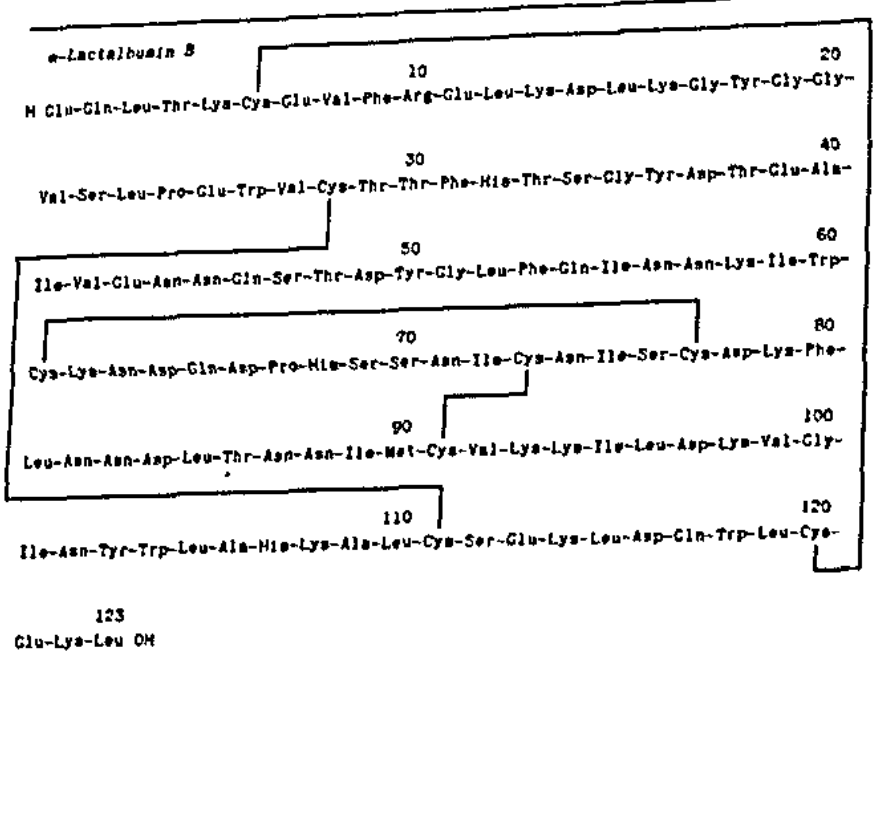
                                                                    90                  100
Asp-Thr-Val-Pro-Ala-Lys-Ser-Cys-Gln-Ala-Gln-Pro-Thr-Thr-Met-Ala-Arg-His-Pro-His-

                                                                    110                 120
Pro-His-Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys-Thr-Glu-Ile-Pro-

                                                                    130                 140
Thr-Ile-Asn-Thr-Ile-Ala-Ser-Gly-Glu-Pro-Thr-Ser-Thr-Pro-Thr-Ile-Glu-Ala-Val-Glu-

                                                                    150                 160
Ser-Thr-Val-Ala-Thr-Leu-Glu-Ala-Ser-Pro-Glu-Val-Ile-Glu-Ser-Pro-Pro-Glu-Ile-Asn-
                                                                     |
                                                                     P
                                                                169
Thr-Val-Gln-Val-Thr-Ser-Thr-Ala-Val OH
```

## Table A.7. (*Continued*)



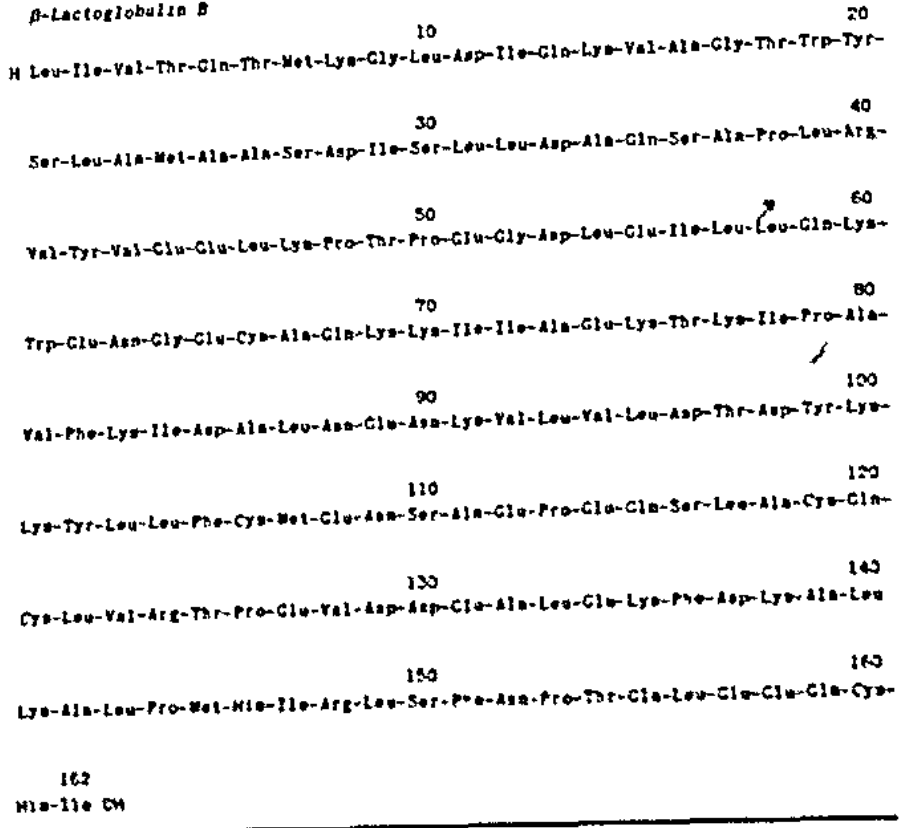



**Table A.8. Location of Amino Acid Substitutions in Genetic Variants of Milk Proteins**

| Protein | Variant | Frequency[a] | Substitutions at Positions | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 14–26 | 53 | | 59 | | 192 | |
| $\alpha_{s1}$-casein | A | R | deleted | | | | | | |
| | B | W | | Ala | | Gln | | Glu | |
| | C | I | | | | | | Gly | |
| | D | R | | ThrP | | | | | |
| | E | R | | | | Lys | | Gly | |
| | | | 18 | 35 | 36 | 37 | 67 | 106 | 122 |
| β-casein | A[1] | | | | | | His | | |
| | A[2] | W, I | Ser P | Ser P | Glu | Glu | Pro | His | Ser |
| | A[3] | | | | | | | Gln | |
| | B | R | | | | | | | Arg |
| | C | R | | Ser | | Lys | | | |
| | D | R | Lys | | | | | | |
| | E | R | | | Lys | | | | |
| | | | 136 | 148 | | | | | |
| κ-casein | A | W, I | Thr | Asp | | | | | |
| | B | X | Ile | Ala | | | | | |
| | | | 10 | | | | | | |
| α-lactalbumin | A | I | Gln | | | | | | |
| | B | W | Arg | | | | | | |
| | | | 45 | 59 | 64 | 118 | | 158 | |
| β-lactoglobulin | A | X | | | Asp | Val | | | |
| | B | W, I | Glu | Gln | Gly | Ala | | Glu | |
| | C | R | | His | | | | | |
| | D | R | Gln | | | | | | |
| | E ($D_{yak}$) | R | | | | | | Gly | |

[a]R = rare; W = predominant in Western cattle; I = predominant in Indian cattle; X = lower frequency than the predominant variant but not rare.

**Table A.9. Various Nonprotein Nitrogenous Compounds in Milk**

| Compound | $mg \cdot kg^{-1}$ |
|---|---|
| *Amines* | |
| 1-propylamine | 3.0–15.0 |
| 1-hexylamine | 5.0–24.0 |
| Ethanolamine | 0.5–8.5[a] |
| Choline | 43.0–285.0[b] |
| Putrescine, Cadaverine | 0.003–0.021 |
| Spermidine | 0.009–0.028 |
| Spermine | 0.006–0.017 |
| *Amino Acids* | |
| Glycine | 2.0–15.0 |
| Alanine | 1.4–2.9 |
| Valine | 0.6–1.5 |
| Leucine | 0.3–0.9 |
| Isoleucine | 0.2–0.9 |
| Serine | 0.8–2.7 |
| Phosphoserine | 0.7 |
| Threonine | 0.8–1.4 |
| Aspartic acid | 0.7–2.9 |
| Asparagine | 1.3 |
| Glutamic acid | 4.0–32.0 |
| Glutamine | 1.8 |
| Lysine | 2.8–8.1 |
| Arginine | 1.4–4.5 |
| Cystine | 0–5.8 |
| Methionine | 0.3–0.7 |
| Phenylalanine | 0.1–0.3 |
| Tyrosine | 0.1–0.5 |
| Histidine | 0.7–5.5 |
| Proline | 2.5–5.4 |
| Hydroxyproline | 0–0.1 |
| Ornithine | 0.3–2.2 |
| Citrulline | 0–13.0 |
| α-aminobutyric acid | 0.1–0.4 |
| *Amino Acid Derivatives* | |
| N-methyl glycine | + |
| Hippuric acid | 31.0–64.0 |
| Salicyluric acid | 0.2 |

**Table A.9. (*Continued*)**

| Compound | mg kg$^{-1}$ |
|---|---|
| Creatine | 80 0 |
| Creatinine | 32 0 |
| Histamine | 0 30–0 05 |
| Phenylacetyl glutamine | > 0 1 |
| Kynurenine | 0 2 |
| Indoxylsulfuric acid | 1 2 |
| Taurine | 1 0–7 0 |
| *Other Compounds* | |
| Carnitine | 10 0–17 0 |
| Acetyl carnitine | 2 0–12 0 |
| Morphine | 0 0002–0 0005 |
| N-acetylneuraminic acid | 120–270[c] |
| N-acetylglucosamine | 11 0 |
| N-acetylglucosamine-1-phosphate | 16 0 |
| NANA-(2→3) Gal-($\beta$1→4)Glc | + |
| NANA-(2→6) Gal-($\beta$1→4)Glc | + |
| NANA-(2→8) NANA-(2→3) Gal-($\beta$1→4)Glc | + |
| NGNA (2→3)-Gal-($\beta$1→4)Glc | ,+ |
| NANA-(2→3) Gal-($\beta$1→4)GlcNAc | + |

[a]See phosphate esters (Table A 2)
[b]Includes about 25 mg kg$^{-1}$ in phospholipids (Table 5 1)
[c]About 3 mg kg$^{-1}$ dialyzable, remainder in protein

**Table A.10. Enzymes in Bovine Milk**

| EC No | Name | Reaction Catalyzed | Site[a] |
|---|---|---|---|
| 1 1 1 27 | Lactate dehydrogenase | L-lactate + $NAD^+$ = pyruvate + NADH + $H^+$ | P |
| 1 1 1 37 | Malate dehydrogenase | L-malate + $NAD^+$ = oxaloacetate + NADH + $H^+$ | |
| 1.2.3 2 | Xanthine oxidase | Xanthine + $H_2O$ + $O_2$ = urate + superoxide | F |
| 1 4 3 6 | Amine oxidase (copper-containing) | $RCH_2NH_2 + H_2O + O_2 = RCHO + NH_3 + H_2O_2$ | |
| 1 6 4 3 | Dihydrolipoamide reductase ($NAD^+$) | NADH + $H^+$ + lipoamide = $NAD^+$ + dehydrolipoamide | F |
| 1 6 99 3 | NADH dehydrogenase (cytochrome C reductase) | NADH + $H^+$ + acceptor = $NAD^+$ + reduced acceptor | F |

**Table A.10. (*Continued*)**

| EC No. | Name | Reaction Catalyzed | Site[a] |
|---|---|---|---|
| 1.8. | Sulfhydryl oxidase[b] | $2\ RSH + O_2 = RS\text{-}SR + H_2O_2$ | S |
| 1.11.1.6 | Catalase | $H_2O_2 + H_2O_2 = O_2 + 2\ H_2O$ | L |
| 1.11.1.7 | Lactoperoxidase | Donor + $H_2O_2$ = oxidized donor + $2\ H_2O$ | S |
| 1.15.1.1 | Superoxide dismutase | $O_2^- + O_2^- + 2\ H^+ = O_2 + H_2O_2$ | |
| 2.3.2.2 | γ-glutamyl transferase | (4-L-glutamyl)-peptide + an amino acid = peptide + 4-L-glutamyl-amino acid | F |
| 2.4.1.22 | Lactose synthase | UDP-galactose + D-glucose = UDP + lactose | S |
| 2.4.99.1 | CMP-N-acetyl-neuraminate-galactosyl-glycoprotein sialyl transferase | CMP-N-acetylneuraminate + D-galactosyl glycoprotein = CMP + N-acetylneuraminyl-D-galactosyl-glycoprotein | |
| 2.6.1.1 | Aspartate amino transferase | L-aspartate + 2-oxoglutarate = oxalacetate + L-glutamate | P |
| 2.6.1.2 | Alanine amino transferase | L-alanine + 2-oxoglutarate = pyruvate + L-glutamate | |
| 2.7.1.26 | Riboflavin kinase | ATP + riboflavin = ADP + FMN | |
| 2.7.1.30 | Glycerol kinase | ATP + glycerol = ADP + *sn*-glycerol-3-phosphate | |
| 2.7.7.2 | FMN adenyltransferase | ATP + FMN = FAD + pyrophosphate | |
| 2.8.1.1 | Thiosulfate sulfur transferase (rhodanese) | $S_2O_3^{2-} + CN^- = SO_3^{2-} + SCN^-$ | |
| 3.1.1.1 | Carboxylesterase | Carboxylic ester + $H_2O$ = alcohol + carboxylate ion | S |
| 3.1.1.2 | Arylesterase | A phenyl acetate + $H_2O$ = a phenol + acetate | |
| 3.1.1.3 | Triacylglycerol lipase[c] | Triacylglycerol + $H_2O$ = diacylglycerol + fatty acid anion | S |
| 3.1.1.7 | Acetylcholine-esterase | Acetylcholine + $H_2O$ = choline + acetate | F |
| 3.1.1.8 | Cholinesterase | An acylcholine + $H_2O$ = choline + carboxylate anion | S |
| 3.1.1.34 | Lipoprotein lipase | As 3.1.1.3 | C |
| 3.1.3.1 | Alkaline phosphatase | An orthophosphoric monoester + $H_2O$ = an alcohol + orthophosphate | F |
| 3.1.3.2 | Acid phosphatase | Same as above | F |
| 3.1.3.5 | 5′-nucleotidase | A 5′-ribonucleotide + $H_2O$ = a ribonucleoside + orthophosphate | F |

**Table A.10. (*Continued*)**

| EC No | Name | Reaction Catalyzed | Site[a] |
|---|---|---|---|
| 3 1 3 9 | Glucose 6 phosphatase | D glucose 6-phosphate + $H_2O$ = D glucose + orthophosphate | F |
| 3 1 3 16 | Phosphoprotein phosphatase ? | Hydrolyzes phosphate ester bonds in phosphoproteins | P |
| 3 1 4 1 | Phosphodiesterase | A phosphoric diester + $H_2O$ = a phosphoric monoester + alcohol | F |
| 3 1 27 5 | Ribonuclease (pancreatic) | Endonucleolytic cleavage to 3′ phosphomono- and oligonucleotides ending in $C_p$ or $U_p$ | S |
| 3 2 1 1 | α-amylase | Hydrolyzes α 1,4 glucan links in polysaccharides containing three or more glucosyl residues | S |
| 3 2 1 2 | β amylase | Hydrolyzes α 1,4 glucan links in polysccharides by removing successive maltose units from nonreducing end | |
| 3 2 1 17 | Lysozyme | Hydrolyzes β-1,4 links between N-acetylmuramic acid and 2-acetamido 2 deoxy D glucose residues in mucopolysaccharides | S |
| 3 2 1 24 | α D mannosidase | Hydrolyzes terminal, nonreducing α D mannose units in α D mannosides | |
| 3 2 1 30 | β-N acetyl D-glucosaminidase | Hydrolyzes terminal, nonreducing 2 acetamido 2 deoxy-β D glucose residues in chitobiose and higher analogs and in proteins | |
| 3 2 1 31 | β-glucuronidase | A β-D glucuronide + $H_2O$ = alcohol + D glucuronate | |
| 3 4 21 7 | Plasmin | Hydrolyzes peptide bonds, preferential cleavage Lys > Arg, higher selectivity than trypsin | C |
| 3 4 | "Acid" proteinase | Hydrolyzes peptide bonds | |
| 3 4 | Lysyl arylamidase? | | |
| 3 6 1 1 | Inorganic pyrophosphatase | Pyrophosphate + $H_2O$ = 2 orthophosphate | |
| 3 6 1 3 | Adenosine triphosphatase ($Mg^{2+}$ activated) | ATP + H O = ADP + orthophosphate | F |
| 3 6 1 9 | Nucleotide pyrophosphatase | A dinucleotide + $H_2O$ = 2 mononucleotides | |

**Table A.10. (*Continued*)**

| EC No. | Name | Reaction Catalyzed | Site[a] |
|---|---|---|---|
| 4.1.2.13 | Fructose-bisphosphate aldolase | D-fructose-1,6-bisphosphate = dihydroxyacetone phosphate + D-glyceraldehyde-3-phosphate | |
| 4.2.1.1 | Carbonic dehydratase | $H_2CO_3 = CO_2 + H_2O$ | |
| 5.3.1.9 | Glucose phosphate isomerase | D-glucose-6-phosphate = D-fructose-6-phosphate | |

*Note:* Milk contains other enzymes, and some enzymes listed may be questionable. Freshly secreted milk contains enzyme systems capable of incorporating fatty acids into glycerides and of desaturating stearic to oleic acid; this system is found in the cytoplasmic droplets in the milk.

[a]Mainly located in plasma (P), serum (S), fat globule membrane (F), casein micelles (C), or leukocytes (L).

[b]Not thiol oxidase, EC 1.8.3.2.

[c]Only present in colostrum, not in normal milk.

**Table A.11. Alcohols, Carbonyls, Acids, and Esters in Milk**

| Class | Compound | Concentration ($mg \cdot kg^{-1}$) |
|---|---|---|
| Alcohols | Ethanol | 3 |
| Aldehydes | Formaldehyde | 0–0.003 |
| | Acetaldehyde | 0–0.016 |
| | Hexanal | + |
| | Benzaldehyde | + |
| Ketones | Acetone | 0–1.1 |
| | Butanone | 0.08 |
| | Diacetyl | + |
| | 2-pentanone | 0.007–0.030 |
| | 2-hexanone | 0.007–0.010 |
| | 2-heptanone | + |
| | 2-nonanone | + |
| Acids | Formic | 10.0–85.0 |
| | Acetic | 3.0–50.0 |
| | Propionic | 0–3.0 |
| | Butyric | 0–9.5 |
| | Valeric | 0–3.8 |
| | Hexanoic | 4.0–10.0 |
| | Octanoic | 12.5–38.0 |
| | Decanoic | + |
| | Lactic | 34.0–104.0 |

**Table A.11. (*Continued*)**

| Class | Compound | Concentration (mg kg$^{-1}$) |
|---|---|---|
| | β-hydroxybutyric | + |
| | δ-decalactone | + |
| | δ-dodecalactone | + |
| | Glyoxylic | + |
| | Pyruvic | 0–25 0 |
| | Acetoacetic | 0–tr |
| | α-ketoglutaric | 0 1–15 0 |
| | Oxalic | 3 0–200 0 |
| | Oxaloacetic | 0–tr |
| | Oxalosuccinic | 0–tr |
| | Citric | 1750 0 |
| | Benzoic | 2 3–4 0 |
| Esters | Ethyl acetate | + |
| | Methyl palmitate | + |

**Table A.12. Nucleic Acids and Nucleotides in Milk**

| Compound | Abbreviation | Concentration (mg kg$^{-1}$) |
|---|---|---|
| Adenosine-5′-monophosphate | AMP | 0 7–21 0 |
| Adenosine-3′,5′-cyclic monophosphate | CAMP | 0 3–9 0 |
| Guanosine-5′-monophosphate | GMP | 0 5 |
| Cytidine-5′-monophosphate | CMP | 1 0–20 0 |
| Uridine-5′-monophosphate | UMP | 4 2–60 0 |
| Adenosine-5′-diphosphate | ADP | 3 0–10 0 |
| Adenosine-5′-triphosphate | ATP | 0 1 |
| Guanosine-5′-diphosphate mannose | GDP Man | 5 4 |
| Guanosine-5′-diphosphate fucose | GDP Fuc | 24 0–40 0 |
| Cytidine -5′-diphosphate choline | CDP Choline | 3 0–12 0 |
| Uridine -5′-diphosphate glucose | UDP-Glc | 4 5–200 0 |
| Uridine -5′-diphosphate galactose | UDP Gal | 4 5–180 0 |
| Uridine -5′diphosphate glucosamine | UDP GlcNAc } | 18 0 |
| Uridine -5′-diphosphte galactosamine | UDP-GalNAc } | |
| Uridine -5′-diphosphate glucuronate | | + ? |
| Nicotinamide adenine dinucleotide | NAD | + |
| Deoxyribonucleic acid | DNA | 11 0–39 0 |
| Ribonucleic acid | RNA | 54 0–176 0 |

**Table A.13. Miscellaneous Compounds Occurring as Conjugates in Milk**

| | |
|---|---|
| Phenol | Methyl vanillate |
| *p*-cresol | Ethyl vanillate |
| 4-ethylphenol | *p*-hydroxyacetophenone |
| 3-n-propylphenol | Benzoic acid |
| 4-allylphenol | Phenylacetic acid |
| Catechol | Hippuric acid |
| 4-methylcatechol | Indole |
| 4-ethylcatechol | Fatty acids |
| Guaiacol | saturated: C4, 6, 8–18 |
| 4-methylguaiacol | unsaturated: 16:1, 18:1, 18:2, 18:3 |
| 4-ethylguaiacol | Lactones of |
| 4-vinylguaiacol | 5-hydroxy $C_{10}$ |
| Vanillin | 5-hydroxy $C_{12}$ |
| Acetovanillone | 4-hydroxy $C_{12}$ |
| Propiovanillone | |

*Note:* Liberated by glucuronidase and arylsulfatase. Source: Brewington et al., *J. Agr. Food Chem.* 22 (1974):293.

## Table A.14. Vitamins in Cow's and Human Milks

| | Content (mg/liter)[a] | | | RDA[b] (mg·day$^{-1}$) | |
|---|---|---|---|---|---|
| | Cow | | | | |
| | Range | Mean | Human | Infant[c] | Adult[d] |
| Vitamin A | 0.1–1.0 | 0.4 | 0.6 | 0.4[e] | 1.00[e] |
| Carotene | 0.03–0.50 | 0.20 | 0.40 | | |
| Vitamin D | 0.0001–0.0012 | 0.0006 | 0.0006 | 0.01 | 0.05 |
| Vitamin E | 0.20–1.84 | 0.98 | 6.64 | 3.00 | 10.00 |
| Thiamin ($B_1$) | 0.20–0.80 | 0.44 | 0.16 | 0.30 | 1.40 |
| Riboflavin ($B_2$) | 0.81–2.50 | 1.75 | 0.36 | 0.40 | 1.60 |
| Niacin | 0.30–2.00 | 0.94 | 1.47 | 6.00 | 18.00 |
| Pantothenic acid | 2.60–4.90 | 3.46 | 1.84 | 2.00 | |
| Vitamin $B_6$ | 0.22–1.90 | 0.64 | 0.10 | 0.30 | 2.20 |
| Biotin | 0.012–0.060 | 0.031 | 0.008 | 0.035 | |
| Folic acid | 0.037–0.072 | 0.050 | 0.050 | 0.030 | 0.40 |
| Vitamin $B_{12}$ | 0.0024–0.0074 | 0.0043 | 0.0003 | 0.0005 | 0.003 |
| Vitamin C | 16.5–27.5 | 21.1 | 43 | 35 | 60 |
| Choline | 43–218 | 121. | 90. | | |
| *Myo*-inositol | | 50. | 330. | | |
| *p*-aminobenzoic acid | 0.04–0.15 | 0.10 | | | |

[a]Data from compilation of A. M. Hartman and L. P. Dryden (*Fundamentals of Dairy Chemistry*, 2nd ed., Ch. 7), except for folate from M. E. Gregory, *J. Dairy Res.* 42 (1975):42.
[b]U.S. Recommended Daily Allowances.
[c]0–0.5 year.
[d]23–50-year males.
[e]Retinol equivalents (RE), 1 RE = 1 μg retinol = 6 μg β-carotene.

## Table A.15. Composition of Some Milk Products

| | | Milk, Fresh, Whole | Milk, Standardized, Heated[a] | Skim Milk (Separated Milk) | Whey, Sweet, Undiluted | "Half and Half" |
|---|---|---|---|---|---|---|
| Dry matter | (g) | 12.3–13.0 | 12.2–12.8 | 9.0–9.5 | 6.3–7.0 | 18–20 |
| Energy[d] | (kJ) | 265 | 260 | 145 | 105 | 530 |
| Fat | (g) | 3.1–4.0 | 3.0–3.75 | 0.04–0.1 | 0.05–0.4 | 10–12 |
| Crude protein | (g) | 3.1–3.6 | 3.1–3.6 | 3.2–3.7 | 0.85–1.15 | 3.0–3.4 |
| Carbohydrate | (g) | 4.5–4.8 | 4.5–4.8 | 4.7–5.1 | 4.6–5.2 | 4.0–4.5 |
| "Ash" | (g) | 0.7–0.8 | 0.7–0.8 | 0.7–0.8 | 0.5–0.6 | 0.6–0.8 |
| Lactose | (g) | 4.5–4.8 | 4.5–4.8 | 4.7–5.1 | 4.6–5.2 | 4.0–4.5 |
| Lactic acid | (g) | tr | tr | tr | 0.05–0.2 | tr |
| Citric acid | (g) | 0.15–0.21 | 0.15–0.21 | 0.16–0.22 | 0.14–0.17 | ~ 0.17 |
| Casein | (g) | 2.3–2.8 | 2.3–2.8 | 2.4–2.9 | 0.04–0.05 | 2.1–2.6 |
| Whey protein | (g) | 0.55–0.72 | 0.55–0.72 | 0.57–0.75 | 0.6–0.8 | 0.5–0.7 |
| Na | (mg) | 35–50 | 35–50 | 36–52 | 36–51 | 35–50 |
| K | (mg) | 140–155 | 140–155 | 145–160 | 140–160 | 130–150 |
| Ca | (mg) | 115–125 | 115–125 | 120–130 | 40–50 | 100–110 |
| Mg | (mg) | 11–14 | 11–14 | 11–14 | 8–10 | 9–12 |
| Fe | (mg) | 0.03–0.1 | 0.03–0.1 | 0.03–0.2 | 0.1 | 0.03–0.1 |
| Cl | (mg) | 95–110 | 95–110 | 100–110 | 70–120 | ~ 85 |
| P | (mg) | 90–100 | 90–100 | 90–100 | 40–55 | 80–95 |
| S | (mg) | 30–35 | 30–35 | 31–36 | ~ 15 | ~ 30 |
| Thiamin | (mg) | 0.03–0.045 | 0.02–0.035 | 0.03–0.04 | 0.03–0.05 | 0.03–0.04 |
| Riboflavin | (mg) | 0.14–0.20 | 0.14–0.20 | 0.14–0.20 | 0.10–0.16 | 0.10–0.16 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Pyridoxine | (mg) | 0 04–0 06 | 0 03–0 05 | 0 04–0 06 | 0 04–0 07 | 0 03–0 05 |
| Vitamin C | (mg) | 1–2 | 0 5–1 1 | 1–2 | 0 9–1 5 | ~ 1 |
| Vitamin A | (mg) | 0 02–0 05 | 0 02–0 05 | 0 001–0 003 | 0 002–0 004 | 0 05–0 15 |
| Carotenoids | (mg) | 0 02–0 03 | 0 02–0 03 | tr | tr | 0 04–0 06 |
| Cholesterol[f] | (mg) | 10–14 | 10–14 | 2–3 | | ~ 35 |
| Other | | | | | 1–10[g] | |
| Density[d] ($\rho^{20}$) | kg m $^{-3}$ | 1030 | 1030 | 1035 | 1025 | 1020 |

| | | Light Cream | Heavy Cream | Ice Cream | Butter, Unsalted[c] | Butter, Salted[d] |
|---|---|---|---|---|---|---|
| Dry matter | (g) | 26–28 | 41–46 | 36–38 | 84–85 | 84–85 |
| Energy[d] | (kJ) | 850 | 1450 | 800 | 3100 | 3000 |
| Fat | (g) | 19–21 | 35–40 | 10–12 | 82 0–83 5 | 81–83 |
| Crude protein | (g) | 2 5–3 0 | 1 9–2 3 | 3 8–4 5 | 0 3–1 0 | 0 3–1 0 |
| Carbohydrate | (g) | 3 6–4 2 | 2 6–3 2 | 20–21 | 0 2–0 5 | 0 2–0 5 |
| "Ash" | (g) | 0 5–0 7 | 0 4–0 5 | 0 8–0 9 | 0 09–0 11 | 0 5–2 5 |
| Lactose | (g) | 3 6–4 2 | 2 6– 3 2 | 5 5–6 5 | 0 2–0 5 | 0 2–0 5 |
| Lactic acid | (g) | tr | tr | tr | 0 1–0 3 | 0 1–0 3 |
| Citric acid | (g) | ~ 0 15 | ~ 0 13 | ~ 0 23 | 0 005–0 02 | 0 005–0 02 |
| Casein | (g) | 1 9–2 3 | 1 5–1 8 | 2 9–3 4 | 0 3–0 9 | 0 3–0 9 |
| Whey protein | (g) | 0 5–0 6 | 0 4–0 5 | 0 7–0 9 | 0 01–0 05 | 0 01–0 05 |
| Na | (mg) | 35–45 | 30–40 | 40–100 | 5–15 | 200–1000 |
| K | (mg) | 120–130 | 80–100 | 100–200 | 10–20 | 10–20 |
| Ca | (mg) | 95–105 | 65–90 | 130–160 | 10–20 | 10–20 |
| Mg | (mg) | 6–11 | 5–8 | 13–17 | 1–3 | 1–3 |
| Fe | (mg) | 0 03–0 1 | 0 03–0 1 | 0 1–0 2 | 0 05–0 2 | 0 05–0 2 |

**Table A.15. (*Continued*)**

| | | Light Cream | Heavy Cream | Ice Cream | Butter, Unsalted[c] | Butter, Salted[d] |
|---|---|---|---|---|---|---|
| Cl | (mg) | ~ 80 | ~ 60 | ~ 130 | 10–25 | 300–1600 |
| P | (mg) | 70–80 | 35–65 | 110–130 | 15–25 | 15–25 |
| S | (mg) | ~ 25 | ~ 20 | ~ 40 | ~ 10 | ~ 10 |
| Thiamin | (mg) | 0.03 | 0.02–0.03 | 0.04–0.06 | 0.003–0.01 | 0.003–0.01 |
| Riboflvin | (mg) | 0.10–0.15 | 0.09–0.12 | 0.1–0.2 | 0.01–0.08 | 0.01–0.08 |
| Pyridoxine | (mg) | 0.02–0.04 | 0.02–0.03 | 0.02–0.04 | ~ 0.005 | ~ 0.005 |
| Vitamin C | (mg) | ~ 1 | 0.6–1 | 0–1 | 0–0.3 | 0–0.3 |
| Vitamin A | (mg) | 0.15–0.25 | 0.3–0.5 | 0.01–0.2 | 0.6–1 | 0.6–1 |
| Carotenoids | (mg) | 0.08–0.12 | 0.15–0.25 | ~ 0.05 | 0.2–0.6 | 0.2–0.6 |
| Cholesterol[f] | (mg) | 60–70 | 100–130 | ~ 35 | 230–260 | 230–260 |
| Other | | | 0–0.5[h] | 0.2–0.5[i]/0.3–1[i] | ~0.15[j] | ~ 0.15[j] |
| Density[d] ($\rho^{20}$) | $kg \cdot m^{-3}$ | 1009 | 988 | ~ 500 | | |

| | | Evaporated Milk (American Standard) | Evaporated Milk (British Standard) | Sweetened Condensed Milk | Whole Milk Powder | Skim Milk Powder |
|---|---|---|---|---|---|---|
| Dry matter | (g) | 25.3–26.3 | 31–33 | 72–75 | 96–98 | 95–97 |
| Energy[d] | (kJ) | 550 | 700 | 1350 | 2100 | 1500 |
| Fat | (g) | 7.5–8.0 | 9–10 | 8–10 | 25–28 | 0.7–1.3 |
| Crude protein | (g) | 6.5–7.1 | 8–9 | 7.5–9.5 | 25–27 | 35–37 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Carbohydrate | (g) | 9–10 | 11.0–12.5 | 53–55 | 36–38 | 49–52 |
| "Ash" | (g) | 1.3–1.6 | 1.9–2.1 | 1.8–2.2 | 6.0–7.0 | 7.5–8.0 |
| Lactose | (g) | 9–10 | 11.0–12.5 | 10.0–12.5 | 36–38 | 49–52 |
| Lactic acid | (g) | tr | tr | tr | tr | tr |
| Citric acid | (g) | 0.33–0.37 | 0.42–0.48 | ~ 0.45 | 1.3–1.4 | 1.8–2.1 |
| Casein | (g) | 4.8–5.4 | 5.9–6.9 | 5.5–6.9 | 18.5–20.5 | 26–28 |
| Whey protein | (g) | 1.1–1.3 | 1.4–1.7 | 1.3–1.7 | 4.4–5.2 | 6.1–7.0 |
| Na | (mg) | 90–140 | 100–180 | 90–150 | 370–420 | 400–550 |
| K | (mg) | 290–320 | 370–420 | 320–380 | 1150–1350 | 1550–1750 |
| Ca | (mg) | 240–280 | 280–320 | 260–300 | 900–1000 | 1200–1300 |
| Mg | (mg) | 25–30 | 30–35 | 25–30 | 85–100 | 110–140 |
| Fe | (mg) | 0.09–0.2 | 0.1–0.2 | 0.1–0.3 | 0.4–0.9 | 0.3–1.1 |
| Cl | (mg) | 200–220 | 270–330 | ~ 250 | 750–800 | ~ 1100 |
| P | (mg) | 190–210 | 200–250 | 210–250 | 700–770 | 950–1050 |
| S | (mg) | 60–70 | 80–90 | ~ 85 | 240–260 | ~ 330 |
| Thiamin | (mg) | 0.04–0.08 | 0.06–0.08 | 0.07–0.1 | 0.2–0.3 | 0.3–0.45 |
| Riboflavin | (mg) | 0.3–0.4 | 0.4–0.5 | 0.3–0.5 | 1.0–1.5 | 1.6–2.2 |
| Pyridoxine | (mg) | 0.05–0.07 | 0.04–0.08 | 0.04–0.1 | 0.2–0.4 | 0.3–0.5 |
| Vitamin C | (mg) | 1–2 | 1–3 | 1–4 | 2–10 | 2–10 |
| Vitamin A | (mg) | 0.05–0.1 | 0.06–0.12 | 0.04–0.14 | 0.2–0.3 | 0.005–0.02[e] |
| Carotenoids | (mg) | 0.03–0.05 | 0.04–0.06 | 0.03–0.05 | 0.15–0.25 | tr |
| Cholesterol[f] | (mg) | ~ 25 | ~ 35 | 25–35 | 80–120 | ~ 20 |
| Density[d] ($\rho^{20}$) | $kg \cdot m^{-3}$ | 1066 | 1085 | 1310 | | |

**Table A.15. (*Continued*)**

| | | Whey Powder | Buttermilk Powder (Sweet) | Buttermilk[b] | Yogurt, Plain | Yogurt, Concentrated |
|---|---|---|---|---|---|---|
| Dry matter | (g) | 94–97 | 96.2–97.2 | 8.5–9.5 | 12–13 | 15–18 |
| Energy[a] | (kJ) | 1450 | 1550 | 150 | 255 | 350 |
| Fat | (g) | 0.8–1.2 | 3–6 | 0.3–0.7 | 3.0–3.75 | 3.5–5.0 |
| Crude protein | (g) | 12–13 | 33–36 | 3.0–3.5 | 3.1–3.6 | 4.2–5.0 |
| Carbohydrate | (g) | 71–73 | 47–49 | 3.5–4.5 | 3.5–4 | 5.0–7.0 |
| "Ash" | (g) | 7.5–8.5 | 7.0–8.0 | 0.7–0.75 | 0.7–0.8 | 0.8–1.0 |
| Lactose | (g) | 71–73 | 47–49 | 3.5–4.5 | 2.5–3 | 4.0–6.0 |
| Lactic acid | (g) | 0.2–2 | tr | 0.5–0.9 | 0.8–1.1 | 0.9–1.2 |
| Citric acid | (g) | ~ 2.2 | 1.5–2.5 | 0.03–0.08 | 0.15–0.2 | ~ 0.24 |
| Casein | (g) | 0.60–0.65 | 25–27 | 2.1–2.5 | 2.3–2.8 | 3.1–3.7 |
| Whey protein | (g) | 8.2–9.0 | 6.0–6.5 | 0.55–0.72 | 0.6–0.7 | 0.7–0.9 |
| Na | (mg) | ~ 600 | 380–530 | 40–55 | 35–50 | 60–70 |
| K | (mg) | ~ 2100 | 1550–1650 | 145–155 | 140–155 | 190–200 |
| Ca | (mg) | 600–800 | 1200–1260 | 100–115 | 115–125 | 150–180 |
| Mg | (mg) | 90–120 | 120–140 | 12–14 | 11–14 | ~ 15 |
| Fe | (mg) | ~ 1.4 | 0.5–1 | 0.03–0.1 | 0.03–0.1 | 0.1–0.2 |
| Cl | (mg) | ~ 1500 | ~ 1000 | ~ 100 | 95–110 | ~ 140 |
| P | (mg) | 580–600 | 900–1000 | 85–100 | 90–100 | 125–135 |
| S | (mg) | ~ 200 | ~ 350 | ~ 35 | 30–35 | ~ 45 |
| Thiamin | (mg) | 0.4–0.6 | 0.3–0.4 | 0.03–0.04 | 0.03–0.045 | 0.04–0.07 |
| Riboflavin | (mg) | 2.3–2.5 | 1.5–2.5 | 0.15–0.18 | 0.14–0.18 | 0.15–0.30 |
| Pyridoxine | (mg) | 0.4–0.6 | 0.2–0.4 | ~ 0.04 | 0.03–0.05 | ~ 0.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Vitamin C | (mg) | ~ 5 | 1–6 | 0.5–1 | 0.5–1 | ~ 1 |
| Vitamin A | (mg) | ~ 0.02 | ~ 0.07 | 0.002–0.007 | 0.02–0.04 | 0.02–0.05 |
| Carotenoids | (mg) | tr | ~0.03 | 0.005–0.01 | ~ 0.02 | ~ 0.02 |
| Cholesterol[f] | (mg) | | ~ 70 | ~ 5 | 10–14 | ~ 15 |
| Other | | 10–150[g] | | ~ 0.5[j] | ~ 0.05[j] | |
| Density[d] ($\rho^{20}$) | $kg \cdot m^{-3}$ | | | 1029 | 1030 | 1040 |

| | | Ymer[c] | Quarg, low-fat | Cottage cheese, creamed |
|---|---|---|---|---|
| Dry matter | (g) | 14–16 | 18–22 | 21–25 |
| Energy[d] | (kJ) | 300 | 330 | 430 |
| Fat | (g) | 3.0–3.5 | 0.2–0.5 | 4–5 |
| Crude protein | (g) | ~ 6.5 | 13–17 | 12.5–14.5 |
| Carbohydrate | (g) | ~ 3.5 | 2.0–4.0 | 1.5–3.5 |
| "Ash" | (g) | ~ 0.9 | 0.9–1.3 | 0.8–1.0 |
| Lactose | (g) | ~ 3.5 | 2.0–4.0 | 1.5–3.5 |
| Lactic acid | (g) | ~ 1 | 0.7–0.8 | 0.5? |
| Citric acid | (g) | ~ 0.04 | 0.05–0.13 | ~ 0.05 |
| Casein | (g) | ~ 4.9 | 12–16 | 12–14 |
| Whey protein | (g) | ~ 1.2 | ~ 0.6 | ~ 0.3 |
| Na | (mg) | ~ 45 | 35–40 | 250–450 |
| K | (mg) | ~ 145 | 90–100 | 50–90 |
| Ca | (mg) | ~ 190 | 80–100 | 60–100 |
| Mg | (mg) | ~ 15 | ~ 12 | 5–6 |
| Fe | (mg) | ~ 0.05 | 0.2–0.4 | 0.1–0.3 |

**Table A.15. (*Continued*)**

| | | Ymer[c] | Quarg, low-fat | Cottage cheese, creamed |
|---|---|---|---|---|
| Cl | (mg) | ~ 105 | 140–150 | 400–700 |
| P | (mg) | ~ 140 | 160–200 | 100–150 |
| S | (mg) | ~ 50 | ~ 140 | ~ 125 |
| Thiamin | (mg) | ~ 0.03 | 0.03–0.04 | 0.02–0.05 |
| Riboflavin | (mg) | ~ 0.2 | 0.2–0.3 | 0.2–0.3 |
| Pyridoxine | (mg) | ~ 0.05 | 0.01–0.1 | 0.01–0.07 |
| Vitamin C | (mg) | ~ 1.5 | ~ 0.5 | tr? |
| Vitamin A | (mg) | ~ 0.04 | 0.002–0.01 | 0.03–0.1 |
| Carotenoids | (mg) | ~ 0.02 | ~ 0.001 | 0.02–0.03 |
| Cholesterol[f] | | | ~ 5 | ~ 15 |
| Other | | | 0-0.5[i]/0-2[k] | |
| Density[d] ($\rho^{20}$) | $kg \cdot m^{-3}$ | 1040 | 1070 | |

*Note:* Approximate composition of products as usually encountered by the user. Quantities per 100 g of product. From numerous sources. Excluding milk of Zebus and Channel Island breeds.

[a] Heating may range from low-pasteurization to sterilization in bottles.
[b] Made from cultured cream.
[c] Made from ultrafiltered milk.
[d] Typical value, assuming the available energy to be 37 k2 per g fat, 17 for protein, 16.8 for lactose, 15 for lactate and 10 for citrate.
[e] Skim milk powder may have been fortified with 0.8°–1.5° mg vitamin A and ~ 0 01 mg vitamin D per 100 g.
[f] Including cholesteryl esters.
[g] Nitrate (mg $NO_3^-$).
[h] Emulsifiers, e.g., monoglycerides (g).
[i] Vegetable gums (g).
[j] Diacetyl (mg).
[k] Modified starch (g).

## Table A.16. Formula and Properties of Milk Components

**Acetal Phospholipids**
See Plasmalogens.

**Acetaldehyde (Ethanal)** $(C_2H_4O)$
$H_3C—CHO$
MW 44.1 BP 21 MP −121 SE ∞ SW ∞

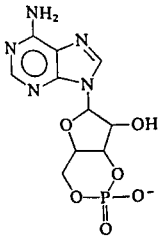
**Acetic Acid** $(C_2H_4O_2)$
$H_3C—CO_2H$
MW 60.1 p*K* 4.75 BP 118 MP 17 SE ∞ SW ∞

**Acetoacetic Acid (3-Oxobutyric Acid)** $(C_4H_6O_3)$
$H_3C—CO—CH_2—CO_2H$
MW 102.1 p*K* 3.6 BP < 100 SE ++ SW ∞

**Acetone** $(C_3H_6O)$
$H_3C—CO—CH_3$
MW 58.1 BP 56 MP −95 SE ∞ SW ∞

**N-Acetylneuraminic Acid = NANA (0-Sialic Acid)** $(C_{11}H_{19}O_9N)$
See Figure 6.5.
MW 309 MP 186

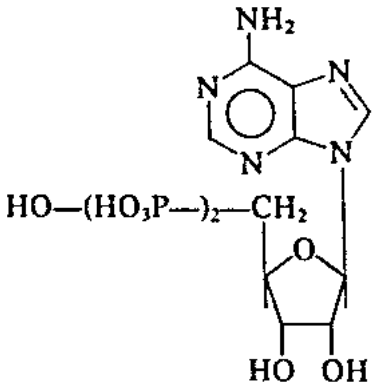
**Adenosine-3′,5′-Cyclic Monophosphate (CAMP)**



$(C_{10}H_{11}O_6PN_5)$
MW 328.2

**Table A.16. (*Continued*)**

**Adenosine-5′-Diphosphate (ADP)**



$(C_{10}H_{15}O_{10}P_2N_5)$

MW 427.2

**Adenosine-5′-Monophosphate (AMP)**

$(C_{10}H_{14}O_7PN_5)$

MW 347.2 p*K* 3.7 ; 6.5 ; 13.1 MP 192

**Adenosine-5′-Triphosphate (ATP)**

$(C_{10}H_{16}O_{13}P_3N_5)$

MW 507.2 SW + +

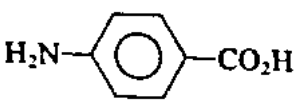
**L-Alanine (Ala)**

$H_3C—CH(—NH_2)—CO_2H$

$(C_3H_7O_2N)$

MW 89.1 p*K* 2.3 ; 9.7 MP 295 SE – SW 15

***p*-Aminobenzoic Acid**



$(C_7H_7O_2N)$

MW 137.1 p*K* 2.5 ; 4.9 MP 187 SE 8 SW 0.5

**α-Aminobutyric Acid**

$H_3C—CH_2—CH(—NH_2)—CO_2H$

$(C_4H_9O_2N)$

MW 103.1 p*K* 2.5 ; 9.7 MP 292 SE – SW + + +

**2-Aminoethanol**

See Ethanolamine.

**Aneurin**

See Thiamin.

**L-Arginine (Arg)**

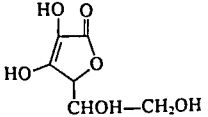
$H_2N—C(=NH)—NH(—CH_2)_3—CH(—NH_2)—CO_2H$

$(C_6H_{14}O_2N_4)$

MW 174.2 p*K* 2.0 ; 9.0 ; 12.5 MP 240 ? SE – SW 15

Table A.16. (*Continued*)

**L-Ascorbic Acid (Vitamin C)** ($C_6H_8O_6$)



MW 176.1 p*K* 4.1 ; 11.8 MP 191 SE – SW 33

**L-Asparagine (Asn)** ($C_4H_8O_3N_2$)

$H_2N—CO—CH_2—CH(—NH_2)—CO_2H$

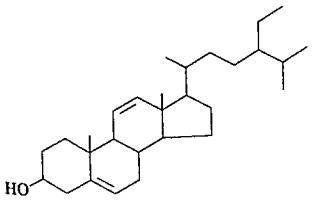
MW 132.1 p*K* 2.1 ; 8.8 MP 235 SE – SW 2

**L-Aspartic Acid (Asp)** ($C_4H_7O_4N$)

$HO_2C—CH_2—CH(—NH_2)—CO_2H$

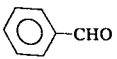
MW 133.1 p*K* 2.1 ; 9.8 ; 3.9 MP 270 ? SE – SW 0.5

**$\Delta^5$-Avenasterol** ($C_{29}H_{48}O$)



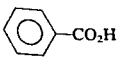
MW 412 MP 137

**Benzaldehyde** ($C_7H_6O$)



MW 106.1 MP −26 BP 178 SE ∞ SW 0.3

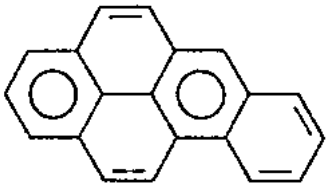
**Benzoic Acid** ($C_7H_6O_2$)



MW 122.1 p*K* 4.2 BP 249 MP 122 SE +++ SW 0.4

Table A.16. (*Continued*)

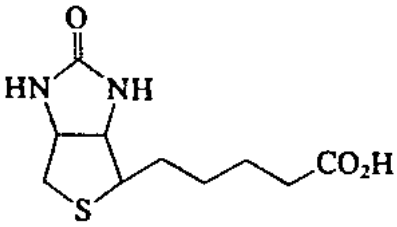
**3,4-Benz(o)pyrene**



$(C_{20}H_{12})$

MW 252.3 MP 177 BP ~470 SW –

**Biotin**




$(C_{10}H_{16}O_3N_2S)$

MW 244.3 MP 232 SE tr SW +

**2-Butanone**

$H_3C—CH_2—CO—CH_3$

MW 72.1 MP −86 BP 80 SE ∝ SW 30

$(C_4H_8O)$

**Butyric Acid**

$H_3C—CH_2—CH_2—CO_2H$

MW 88.1 p*K* 4.8 MP −6 BP 163 SE ∝ SW ∝

$(C_4H_8O_2)$

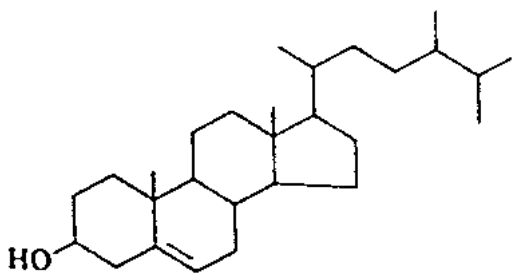
**Cadaverine (1,5-Diaminopentane)**

$H_2N(—CH_2)_5—NH_2$

MW 102.2 BP 179 MP 9 SE tr SW + +

$(C_5H_{14}N_2)$

**Campesterol**




$(C_{28}H_{48}O)$

MW 400 MP 158 SE + + SW 0

**Capric Acid (Decanoic Acid)**

$H_3C(—CH_2)_6—CO_2H$

MW 172.3 p*K* 4.8 BP 270 MP 32 SE + + SW tr

$(C_{10}H_{20}O_2)$

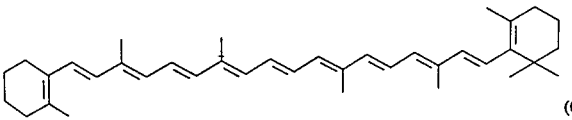
**Table A.16. (*Continued*)**

**Caproic Acid (Hexanoic Acid)**
$H_3C(-CH_2)_4-CO_2H$ $(C_6H_{12}O_2)$
MW 116 2 p$K$ 4 9 BP 205 MP −2 SE + + SW tr

**Caprylic Acid (Octanoic Acid)**
$H_3C(-CH_2)_6-CO_2H$ $(C_8H_{16}O_2)$
MW 144 2 p$K$ 4 9 BP 238 MP 16 SE + + SW tr

**Carnitine (Vitamin $B_T$)**
$(H_3C-)_3N^+-CH_2-CHOH-CH_2-COO^-$ $(C_7H_{15}O_3N)$
MW 161 2 MP 196 SW + +

**β-Carotene**

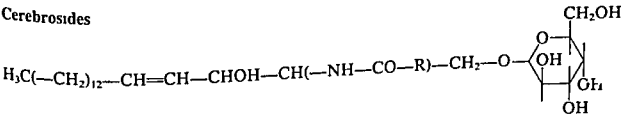


$(C_{40}H_{56})$

MW 536 9 MP 182 SE + SW −

**Cephalin**
See Phosphatidylethanolamine

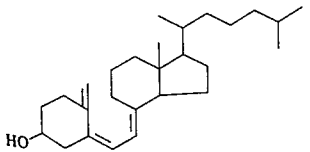
**Cerebrosides**



$(C_{48}H_{93}NO_8)$

e g , Galactocerebroside
MW 811 MP 182

**Cholecalciferol (Vitamin $D_3$)**

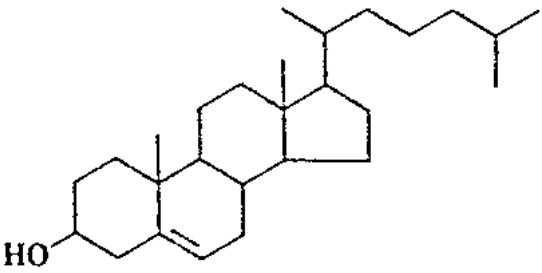


$(C_{27}H_{44}O)$

MW 384 5 MP 86 SE + SW −

**Table A.16. (*Continued*)**

**Cholesterol**



$(C_{27}H_{46}O)$

MW 386.6 BP 360 MP 149 SE 18 SW 0.26

**Choline**

$HOH_2C—CH_2—N(—CH_3)_3—OH$ $(C_5H_{15}O_2N)$

MW 121.2 p*K* 13.9 SE – SW +++

**Citric Acid**

$HO_2C—CH_2—COH(—CO_2H)—CH_2—CO_2H$ $(C_6H_8O_7)$

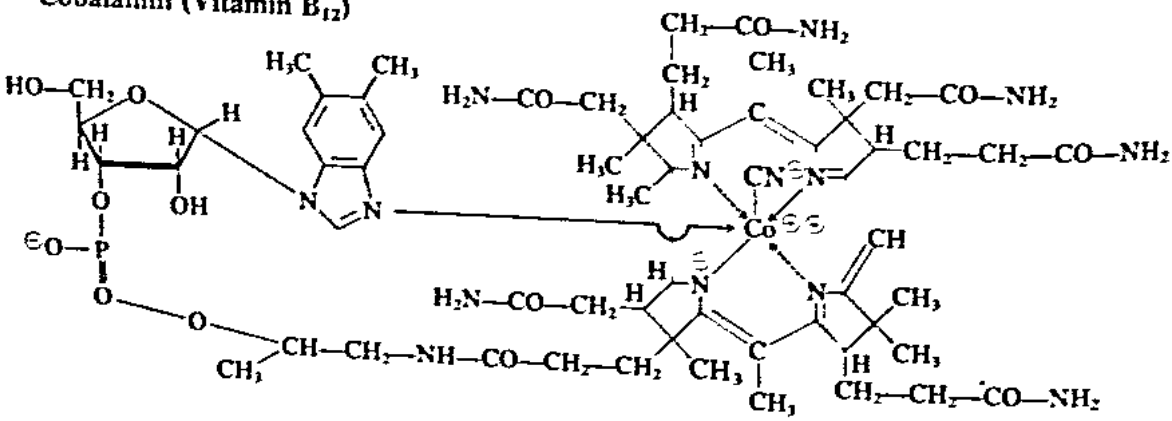
MW 192.1 p*K* 3.1 ; 4.7 ; 5.4 MP 153 SE 2.3 SW 133

**L-Citrulline**

$H_2N—CO—NH(—CH_2)_3—CH(—NH_2)—CO_2H$ $(C_6H_{13}O_3N_3)$

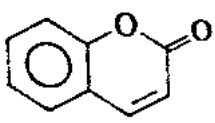
MW 175.2 p*K* 2.4 ; 9.4 MP 220 SW ++

**Cobalamin (Vitamin $B_{12}$)**



MW 1355.4 SE – SW 1.3 $C_{63}H_{88}O_{14}PN_{14}Co$

**Coumarin (1,2-Benzopyrone)**



$(C_9H_6O_2)$

MW 146.1 BP 290 MP 70 SE ++ SW 0.01

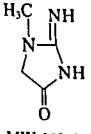
Table A.16. (*Continued*)

**Creatine** ($C_4H_9O_2N_3$)

$H_2N—C(=NH)—N(—CH_3)—CH_2—CO_2H$

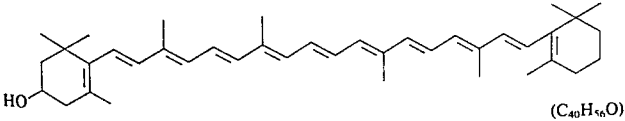
MW 131.1 p*K* 2.7 ; 11.0 MP 300 SE tr SW 1.4

**Creatinine** ($C_4H_7ON_3$)



MW 113.1 p*K* 4.8 ; 9.2 MP 300 SE – SW 8.7

**Cryptoxanthin** ($C_{40}H_{56}O$)



MW 552.9 MP 169 SW –

**Cyanocobalamin**

See Cobalamin.

**L-Cysteine (Cys)** ($C_3H_7O_2NS$)

$HO_2C—CH(—NH_2)—CH_2SH$

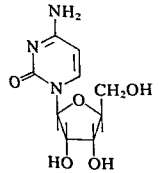
MW 121.2 p*K* 1.7 ; 8.3 ; 10.8 MP 240 SE – SW + +

**L-Cystine** ($C_6H_{12}O_4N_2S_2$)

$[HO_2C—CH(—NH_2)—CH_2S—]_2$

MW 240.3 p*K* 1.7 ; 2.3 ; 7.9 ; 9.9 MP 260 SE – SW 0.01

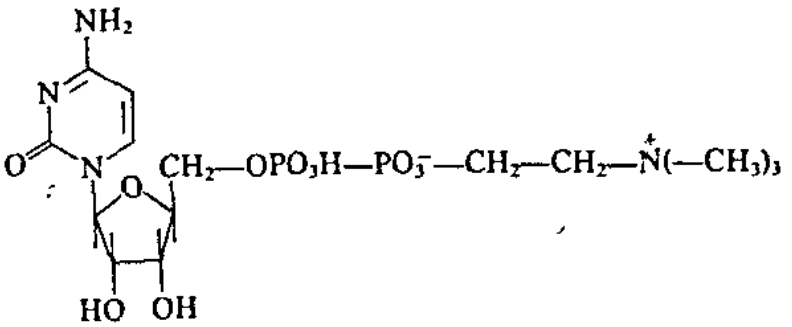
**Cytidine** ($C_9H_{13}O_4N_3$)



MW 243.2 p*K* 4.1 ; 12.3 MP 230 SW + +

Table A.16. (*Continued*)

**Cytidine-5′-Diphosphate Choline (CDP-Choline)**



$(C_{13}H_{24}O_{11}P_2N_4)$

MW 474

**Cytidine-5′-Monophosphate (CMP)**

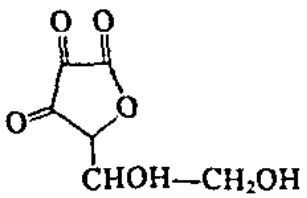
$(C_9H_{14}O_8PN_3)$

MW 323.2 p*K* 4.5 ; 6.3 MP 233

**Decanoic Acid**

See Capric acid.

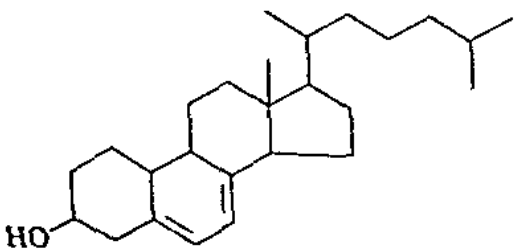
**Dehydro-L-Ascorbic Acid (Vitamin C)**



$(C_6H_6O_6)$

MW 174.1 MP 220 ? SE − SW + +

**7-Dehydrocholesterol (Provitamin $D_3$)**

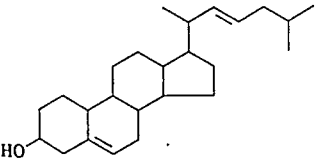


$(C_{27}H_{44}O)$

MW 384.7 MP 150 SE + + SW −

Table A.16. (*Continued*)

**22-Dehydrocholesterol** $(C_{27}H_{44}O)$



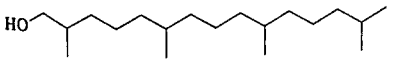
MW 384.6 MP 135

**Diacetyl (Biacetyl)** $(C_4H_6O_2)$

$H_3C—CO—CO—CH_3$

MW 86.1 BP 88 MP −2 SE ∞ SW + + +

**Dihydrophytol** $(C_{20}H_{42}O)$



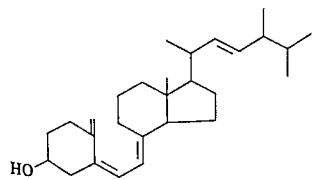
MW 298.5

**Dimethyl Sulfide (Methyl Sulfide)** $(C_2H_6S)$

$H_3C—S—CH_3$

MW 62.1 BP 38 MP −98 SE + + SW −

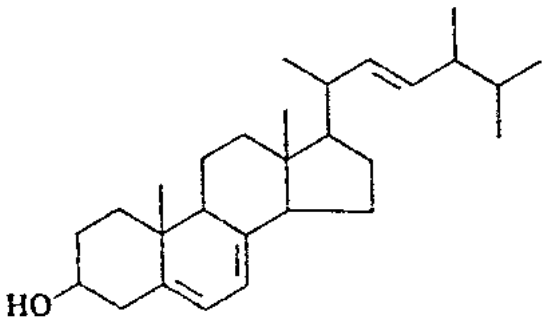
**Ergocalciferol (Vitamin $D_2$)** $(C_{28}H_{44}O)$



MW 396.7 MP 116 SE + + SW −

Table A.16. (*Continued*)

Ergosterol (Provitamin $D_2$)



$(C_{28}H_{44}O)$

MW 396.7 MP 168 SE + SW –

Ethanol (Ethyl Alcohol)

$H_3C—CH_2OH$

MW 46.1 BP 78 MP –117 SE ∝ SW ∝ $(C_2H_6O)$

Ethanolamine (2-Aminoethanol, Colamine)

$H_2N—CH_2—CH_2OH$

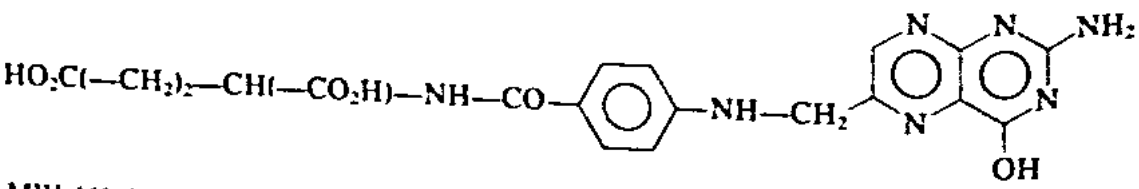
MW 61.1 p*K* 9.5 BP 170 MP 10 SW ∝ $(C_2H_7ON)$

Ethyl Acetate

$H_3C—CO—OCH_2—CH_3$

MW 88.1 BP 77 MP –83 SE ∝ SW + + $(C_4H_8O_2)$

Folic Acid (Pteroylglutamic Acid)



MW 441.4 MP 250 SE – SW tr $(C_{19}H_{19}O_6N_7)$

Formaldehyde (Methanal)

HCHO

MW 30 0 BP –21 MP –92 SE ∝ SW + + + $(CH_2O)$

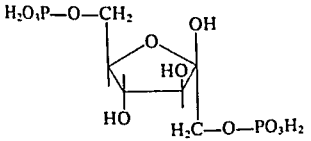
Formic Acid (Methanoic Acid)

$HCO_2H$

MW 46 0 p*K* 3.7 BP 101 MP 8 SE ∝ SW ∝ $(CH_2O_2)$

Table A.16. (*Continued*)

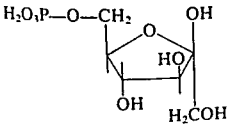
**Fructose-1,6-Diphosphate (FDP)**



$(C_6H_{14}O_{12}P_2)$

MW 340

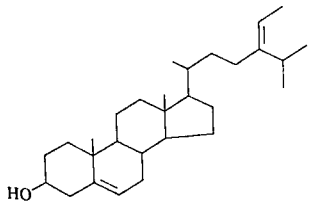
**Fructose-6-Phosphate**



$(C_6H_{13}O_9P)$

MW 260 p*K* 1 , 6 1

**Fucosterol (24-Ethylidenecholest-5-en-3$\beta$-ol)**



$(C_{29}H_{48}O)$

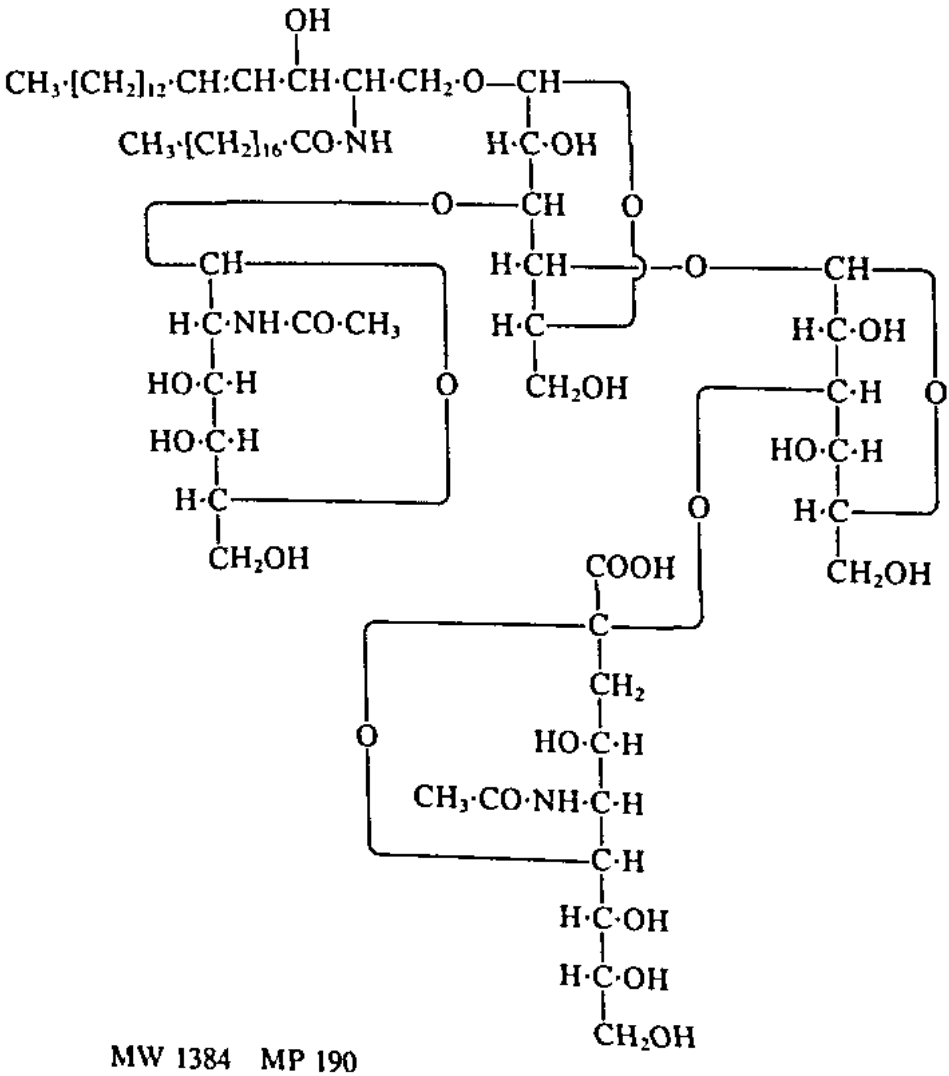
MW 412 MP 124

**Galactose-1-Phosphate**

See Figure 3 1.

**Table A.16. (*Continued*)**

Gangliosides, e.g., ganglioside $G_2$



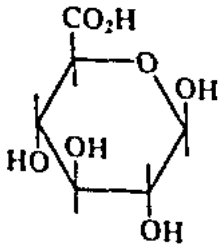
$(C_{67}H_{122}O_{26}N_3)$

MW 1384 MP 190

**α-Glucose-1-Phosphate (Cory's Ester)**
See Figure 3.1.
MW 260.1 p*K* 1.1 ; 6.3

**Glucose-6-Phosphate**
See Figure 3.1.
MW 260.1 p*K* 0.9 ; 6 1

**Glucuronic Acid**



$(C_6H_{10}O_7)$

MW 194.1 MP 165 SW + +

**Table A.16. (*Continued*)**

**L-Glutamic Acid (Glu)** $(C_5H_9O_4N)$

$HO_2C—CH_2—CH_2—CH(—NH_2)—CO_2H$

MW 147.1 p*K* 2.2 ; 9.7 ; 4.3 MP 225 SE tr SW 1

**L-Glutamine (Gln)** $(C_5H_{10}O_3N_2)$

$H_2N—CO—CH_2—CH_2—CH(—NH_2)—CO_2H$

MW 146.1 p*K* 2.2 ; 9.2 MP 185 SE – SW 4

**Glycerol-1-Phosphate (Glycerophosphoric Acid)** $(C_3H_9O_6P)$

$H_2OHC—CHOH—CH_2—O—PO_3H_2$

MW 172.1 p*K* 1.4 ; 6.4 MP –20 SE – SW ∞

**Glycerolphosphoethanolamine** $(C_5H_{14}O_6PN)$

$H_2OHC—CHOH—CH_2—O—PO_2H—O—CH_2—CH_2NH_2$

MW 215.1

**Glycine (Gly)** $(C_2H_5O_2N)$

$H_2N—CH_2—CO_2H$

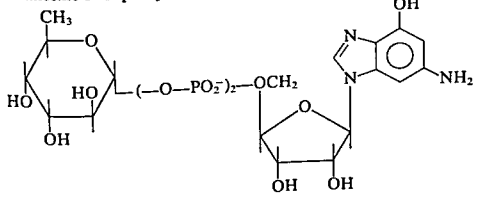
MW 75.1 p*K* 2.3 ; 9.7 MP 260 ? SE – SW 25

**Glyoxylic Acid (aldehydo-formic acid)** $(C_2H_2O_3)$

$OHC—CO_2H$

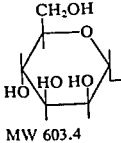
MW 74.0 p*K* 3.3 MP 98 SE tr SW ∞

**Guanosine-5′-Diphosphate Fucose (GDP-Fuc)** $(C_{16}H_{23}O_{15}P_2N_5)$



MW 587.4

**Guanosine-5′-Diphosphate Mannose (GDP-Man)** $(C_{16}H_{23}O_{16}P_2N_5)$




MW 603.4

Table A.16. (*Continued*)

**Guanosine-5′-Monophosphoric Acid (GMP)**
MW 363.2 p*K* 2.4 ; 9.4 ; 6.1 MP 195 ($C_{10}H_{14}O_8PN_5$)

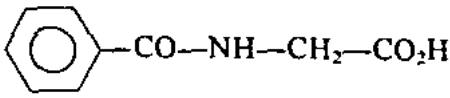
**2-Heptanone**
$H_3C(—CH_2)_4—CO—CH_3$ ($C_7H_{14}O$)
MW 114.2 BP 150 MP −35 SE ++ SW tr ?

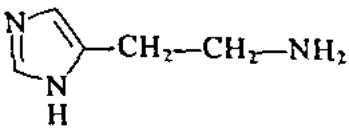
**Hexanal**
$H_3C(—CH_2)_4—CHO$ ($C_6H_{12}O$)
MW 100.2 BP 130 MP −56 SE +++ SW tr

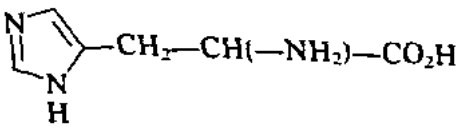
**Hexanoic Acid**
See Caproic acid.

**2-Hexanone**
$H_3C(—CH_2)_3—CO—CH_3$ ($C_6H_{12}O$)
MW 100.2 BP 128 MP −57 SE ∞ SW tr

***n*-Hexylamine (1-Aminohexane)**
$H_3C(—CH_2)_5—NH_2$ ($C_6H_{15}N$)
MW 101.2 p*K* 10.6 BP 130 MP −19 SE ∞ SW tr

**Hippuric Acid**

($C_9H_9O_3N$)
MW 179.2 p*K* 3.8 MP 190 SE tr SW 0.4

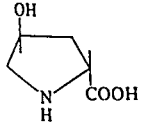
**Histamine**

($C_5H_9N_3$)
MW 111.2 p*K* 6.0 ? MP 83 SE tr SW +++

**L-Histidine (His)**

($C_6H_9O_2N_3$)
MW 155.2 p*K* 1.8 ; 9.2 ; 6.0 MP 280 ? SE − SW 4

***β*-Hydroxybutyric Acid (3-Hydroxybutanoic Acid)**
$H_3C—CHOH—CH_2—CO_2H$
MW 104.1 p*K* 4.5 MP 49 SE ∞ SW ∞ ($C_4H_8O_3$)

Table A.16. (*Continued*)

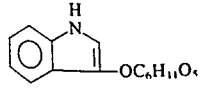
**L-Hydroxyproline (Hyp)**



$(C_5H_9O_3N)$

MW 131.1 p*K* 1.9 ; 9.7 MP 274 SW 35

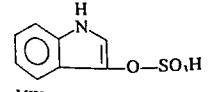
**Indican (Indoxyl-β-Glucoside)**



$(C_{14}H_{17}O_6N)$

MW 295.3 MP 180 SE tr SW + +

**Indoxylsulfate**



$(C_8H_7O_4S)$

MW 213.2 SW +

**Inositol**

See *Myo*-inositol.

**Isocitric Acid** $(C_6H_8O_7)$

$HO_2C—CH_2—CH(—CO_2H)—CHOH—CO_2H$

MW 192.1 MP 105 SE tr SW +

**L-Isoleucine (Ile)** $(C_6H_{13}O_2N)$

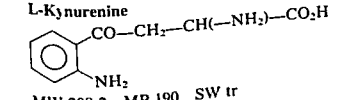
$H_3C—CH_2—CH(—CH_3)—CH(—NH_2)—CO_2H$

MW 131.2 p*K* 2.4 ; 9.7 MP 285 SE – SW 4

**α-Ketoglutaric Acid**

See 2-Oxoglutaric acid.

**L-Kynurenine**



$(C_{10}H_{12}O_3N_2)$

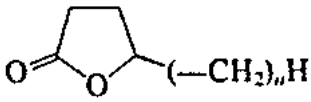
MW 208.2 MP 190 SW tr

**Lactic Acid**

See Table A.18.

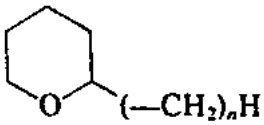
Table A.16. (*Continued*)

**γ-Lactones**



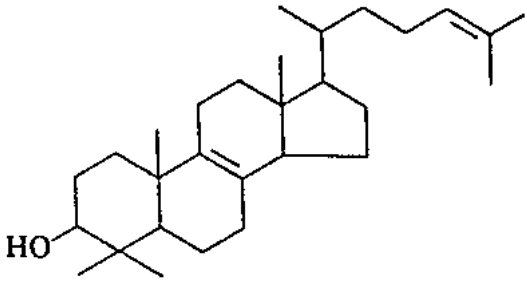
SE + + SW −

**δ-Lactones**



SE + + + SW −

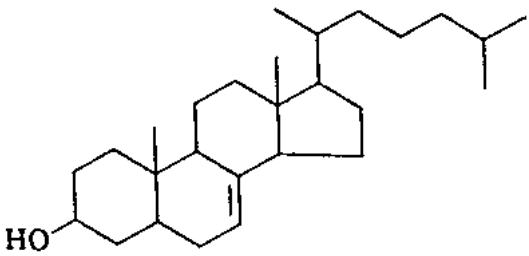
**Lanosterol (Cryptosterol)**



$(C_{30}H_{50}O)$

MW 426.7 MP 140 SE + + +

**Lathosterol**



$(C_{27}H_{46}O)$

MW 386.6 MP 122

**Lecithins (Phosphatidyl Cholines)**

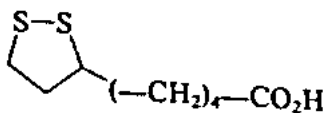
Phosphatidyl$^-$—$CH_2$—$CH_2$—$\overset{+}{N}$(—$CH_3$)$_3$

**L-Leucine (Leu)**

$(H_3C)_2$—CH—$CH_2$—CH(—$NH_2$)—$CO_2H$

MW 131.2 p*K* 2.4 ; 9.7 SE − SW 2 $(C_6H_{13}O_2N)$

**α-Lipoic Acid (6,8-Thioctic Acid)**



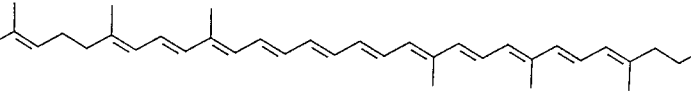
$(C_8H_{14}O_2S_2)$

MW 206.3 p*K* 4.7 MP 47 SE + + SW tr

Table A.16. (*Continued*)

**Lutein**
See Xanthophyll.

**Lycopene**



$(C_{40}H_{56})$

MW 536 MP 175 SE + +

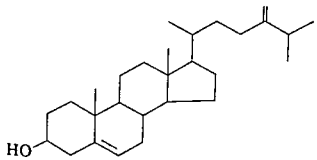
**L-Lysine (Lys)**
$H_2N(—CH_2)_4—CH(—NH_2)—CO_2H$ $(C_6H_{14}O_2N_2)$
MW 146.2 p*K* 2.2 ; 9 ; 10.6 MP 224 SE − SW ∞

**Methanal**
See Formaldehyde.

**L-Methionine (Met)**
$H_3C—S—CH_2—CH_2—CH(—NH_2)—CO_2H$ $(C_5H_{11}O_2NS)$
MW 149.2 p*K* 2.3 ; 9.2 BP 186 MP 283 SE − SW 5

**Methyl Sulfide**
See Dimethyl sulfide.

**24-Methylenecholesterol (24-Methylenecholest-5-en-3$\beta$-ol)**



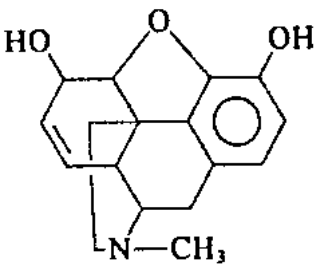

$(C_{28}H_{46}O)$

MW 398.6 MP 143

**N-Methylglycine (Sarcosine)**
$H_3C—NH—CH_2—CO_2H$ $(C_3H_7O_2N)$
MW 89.1 p*K* 2.2 ; 10.2 MP 211 SE − SW + +

Table A.16. (*Continued*)

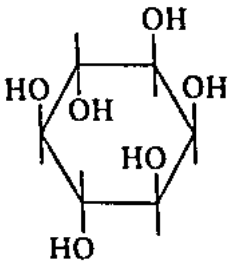
Morphine



$(C_{17}H_{19}O_3N)$

MW 285.3 MP 254 SE tr SW tr

*Myo*-Inositol

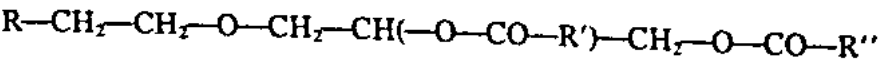


$(C_6H_{12}O_6)$

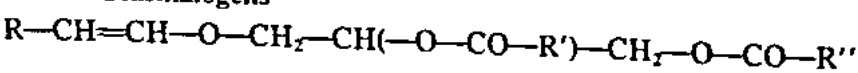
MW 180.2 MP 220 SE – SW 15

NANA = N-Acetylneuraminic Acid
See Figure 6.5.

Neutral Glyceryl Ethers

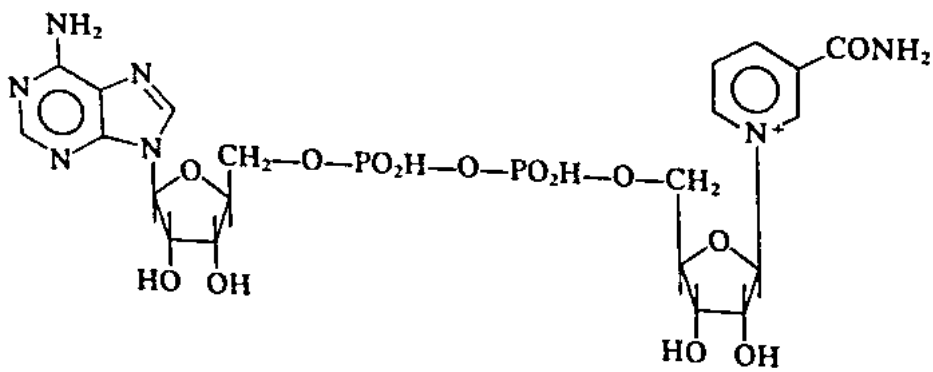


Neutral Plasmalogens



Nicotinamide
See Nicotinic acid amide.

Nicotinamide Adenine Dinucleotide ($NAD^+$)

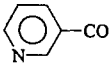


MW 663.4 SW + +

$(C_{21}H_{27}O_{14}P_2N_7)$

**Table A.16. (*Continued*)**

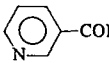
**Nicotinic Acid (Niacin, Pyridine-3-carboxylic acid)**



$(C_6H_5O_2N)$

MW 123.1 p*K* 4.8 MP 236 SE tr SW tr

**Nicotinic Acid Amide (Nicotinamide, Niacinamide)**



$(C_6H_6ON_2)$

MW 122.1 MP 130 SE tr SW + +

**2-Nonanone**

$H_3C(—CH_2)_6—CO—CH_3$ $(C_9H_{18}O)$

MW 142.2 BP 195 MP −8 SE + + SW −

**Octanoic Acid**

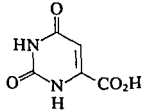
See Caprylic Acid.

**L-Ornithine**

$H_2N(—CH_2)_3—CH(—NH_2)—CO_2H$ $(C_5H_{12}O_2N_2)$

MW 132.2 p*K* 1.8 ; 8.7 ; 10.8 MP 140 SE tr SW + + +

**Orotic Acid (6-Uracilcarboxylic acid)**



$(C_5H_4O_4N_2)$

MW 156.1 p*K* 2.5 , 9.5 ; 13 MP 346 SE − SW +

**Oxalic Acid**

$HO_2C—CO_2H$ $(C_2H_2O_4)$

MW 90.0 p*K* 1.2 ; 4.2 BP 157 MP 189 SE tr SW 10

**Oxaloacetic Acid**

$HO_2C—COH{=}CH—CO_2H$ $(C_4H_4O_5)$

MW 132 p*K* 2.2 ; 4.1

**Oxalosuccinic Acid**

$HO_2C—CO—CH(—CH_2—CO_2H)—CO_2H$ $(C_6H_6O_7)$

MW 190

Table A.16. (*Continued*)

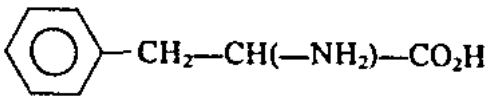
**2-Oxoglutaric Acid (α-Ketoglutaric Acid, 2-Oxopentanedioic Acid)**
$HO_2C—CH_2—CH_2—CO—CO_2H$ $(C_5H_6O_5)$
MW 146.1 MP 115 SE ++ SW ++

**D-Pantothenic Acid**
$HO—CH_2—C(—CH_3)_2—CHOH—CO—NH—CH_2—CH_2—CO_2H$ $(C_9H_{17}O_5N)$
MW 219.2 SE + SW +++

**Pentanoic Acid**
See Valeric acid.

**2-Pentanone**
$H_3C—CH_2—CH_2—CO—CH_3$ $(C_5H_{10}O)$
MW 86.1 BP 102 MP −88 SE ∞ SW tr

**L-Phenylalanine (Phe)**



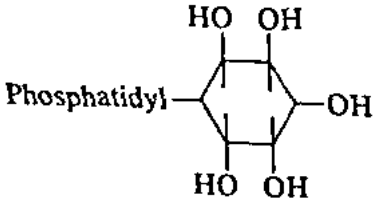
$(C_9H_{11}O_2N)$
MW 165.2 p*K* 2 ; 9 MP 283 SW 3

**Phosphatidic Acids**
$R—CO—O—CH_2—CH(—O—CO—R')—O—PO_3H_2$

**Phosphatidyl Choline**
See Lecithin.

**Phosphatidyl Ethanolamines (Cephalin)**
$Phosphatidyl^-—CH_2—CH_2—\overset{+}{N}H_3$

**Phosphatidyl Inositols**




**Phosphatidyl Serines**
$Phosphatidyl—CH_2—CH(—\overset{+}{N}H_3)—CO_2^-$

**Phosphoethanolamine**
$PO_3H_2—O—CH_2—CH_2—NH_2$
MW 141.1 $(C_2H_8O_4PN)$

**Table A.16. (*Continued*)**

**Phosphopyruvic Acid**

$H_2C{=}C(-OPO_2H)-CO_2H$ $(C_3H_4O_5P)$

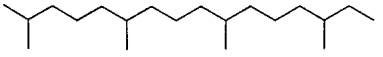
MW 151.0 p*K* 3.5 ; 6.4

**L-Phosphoserine**

$H_2O_3PO-CH_2-CH(-NH_2)-CO_2H$ $(C_3H_8O_6PN)$

MW 185.1

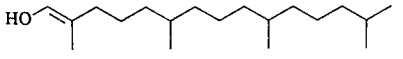
**Phytane (2,6,10,14-Tetramethylhexadecane)**



$(C_{20}H_{42})$

MW 282 SE ++ SW −

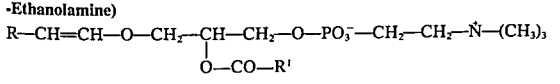
**Phytol (3,7,11,15-Tetramethyl-2-Hexadecen-1-ol)**



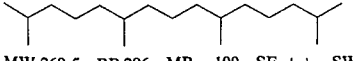
$(C_{20}H_{40}O)$

MW 296.5 SE ∞ SW −

**Plasmalogens (Acetalphospholipids; Phosphatidal-Choline and -Ethanolamine)**



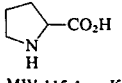
**Pristane (2,6,10,14-Tetramethylpentadecane)**



$(C_{19}H_{40})$

MW 268.5 BP 296 MP −100 SE ++ SW −

**L-Proline (Pro)**



$(C_5H_9O_2N)$

MW 115.1 p*K* 2 ; 10.6 MP 220 SE − SW 162

**Propionic Acid (Propanoic Acid)**

$H_3C-CH_2-CO_2H$ $(C_3H_6O_2)$

MW 74.1 p*K* 4.9 BP 141 MP −20 SE ++ SW ∞

Table A.16. (*Continued*)

**Propylamine (1-Aminopropane)**

$H_3C—CH_2—CH_2—NH_2$ $(C_3H_9N)$

MW 59.1 p*K* 10.6 BP 48 MP −83 SE ++ SW +

**Pteroylglutamic Acid**

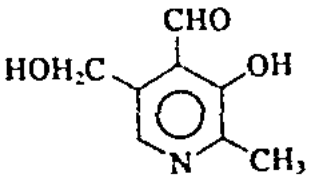
See Folic acid.

**Putrescine**

$H_2N(—CH_2)_4—NH_2$ $(C_4H_{12}N_2)$

MW 88.2 p*K* 11.2 BP 160 MP 27 SW ++

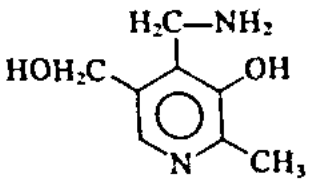
**Pyridoxal (Vitamin $B_6$)**



$(C_8H_9O_3N)$

MW 167.2 p*K* 4.2 ; 8.7 ; 13.0 As hydrochloride· MP 165 SW ++

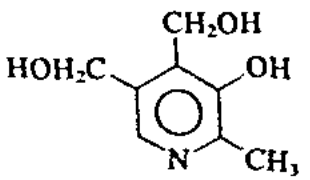
**Pyridoxamine (Vitamin $B_6$)**



$(C_8H_{12}O_2N_2)$

MW 168.2 p*K* 3.3 ; 7.9; 10.4 As dihydrochloride: MP 226 SW ++

**Pyridoxine (Vitamin $B_6$)**



$(C_8H_{11}O_3N)$

MW 169.2 p*K* 5.0 ; 9.0 MP 160 SE tr SW ++ As hydrochloride: SE − SW 20

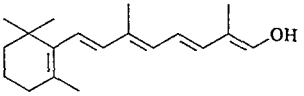
**Pyruvic Acid**

$H_3C—CO—CO_2H$ $(C_3H_4O_3)$

MW 88.1 p*K* 2.5 BP 165 MP 14 SE +++ SW ∞

**Table A.16. (*Continued*)**

**Retinol (Vitamin $A_1$)**




$(C_{20}H_{30}O)$

MW 286.5 MP 63 SE ++ SW −

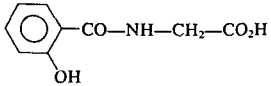
**Riboflavin (Vitamin $B_2$)**

See Figure 11.7

$(C_{17}H_{20}O_6N_4)$

MW 376.4 p*K* 10 MP 280 SE − SW 0.01

**Salicyluric Acid (2-Hydroxybenzamidoacetic Acid, 2-Hydroxyhippuric Acid)**




$(C_9H_9O_4N)$

MW 195 p*K* strong acid MP 170 SE tr SW tr

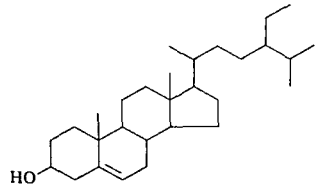
**L-Serine (Ser)**

$HOH_2C—CH(—NH_2)—CO_2H$

$(C_3H_7O_3N)$

MW 105.1 p*K* 2.2 ; 9.2 MP 228 SE − SW 40

***β*-Sitosterol (Cinchol)**




$(C_{29}H_{50}O)$

MW 414.7 MP 140 SE ++

**Spermidine**

$H_2N(—CH_2)_4—NH(—CH_2)_3—NH_2$

$(C_7H_{19}N_3)$

MW 145

**Spermine (Gerontine)**

$H_2N(—CH_2)_3—NH(—CH_2)_4—NH(—CH_2)_3—NH_2$

$(C_{10}H_{26}N_4)$

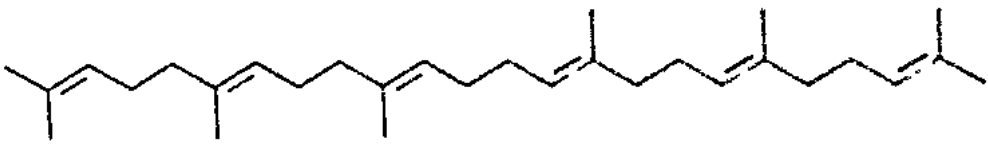
MW 202.3 p*K* strong base

Table A.16. (*Continued*)

**Sphingomyelins**

$H_3C(-CH_2)_{12}-CH=CH-CHOH-CH(-NH-CO-R)-CH_2-O-PO_2^-CH_2-CH_2-\overset{+}{N}(-CH_3)_3$

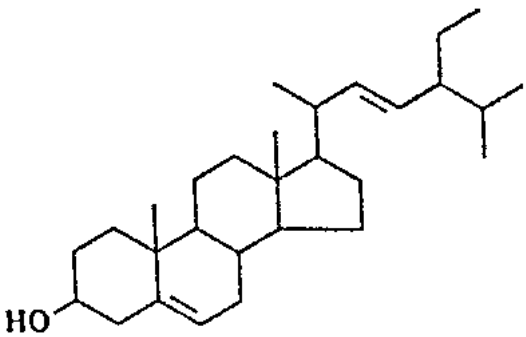
**Squalene (Spinacene; 2,6,10,15,19,23-Hexamethyltetracosane-2,6,10,14,18,22-Hexaene)**



$(C_{30}H_{50})$

MW 410.7 MP < −20 SE + + SW −

**Stigmasterol**



$(C_{29}H_{48}O)$

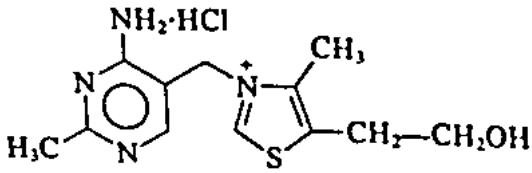
MW 412.7 MP 170 SE + + SW −

**Taurine**

$H_2N-CH_2-CH_2-SO_3H$ $(C_2H_7O_3NS)$

MW 125.2 p*K* 1.5 ; 9 MP 325 SE − SW 10

**Thiamin (Vitamin $B_1$; Aneurin)**



$\cdot Cl$ $(C_{12}H_{18}ON_4Cl_2S)$

MW 337.3 MP 250 SE − SW + + +

**Thiocyanate Ion**

$S=C=N^-$

MW 58.1 p*K* −1.8 SE + + + SW + + +

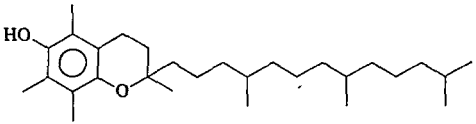
**L-Threonine (Thr)**

$H_3C-CHOH-CH(-NH_2)-CO_2H$

MW 119.1 p*K* 2.1 ; 9.1 MP 253 SW 25 ? $(C_4H_9O_3N)$

**Table A.16. (*Continued*)**

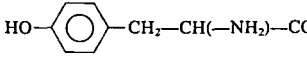
**α-Tocopherol (Vitamin E)**



$(C_{29}H_{50}O_2)$

MW 430.7 BP 350 MP 3 SE ++ SW –

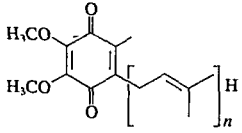
**L-Tyrosine (Tyr)**



$(C_9H_{11}O_3N)$

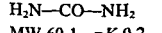
MW 181.2 p*K* 2.2 ; 9.1 ; 10.1 MP 342 SE – SW 0.045

**Ubiquinones (Coenzyme Q)**



$n$ = 6 to 10

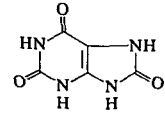
**Urea (Carbamide)**



$(CH_4ON_2)$

MW 60.1 p*K* 0.2 MP 133 SE – SW 110

**Uric Acid**

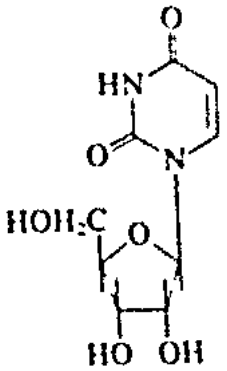


$(C_5H_4O_3N_4)$

MW 168.1 p*K* 3.9 ; 5.6 SE – SW tr

Table A.16. (*Continued*)

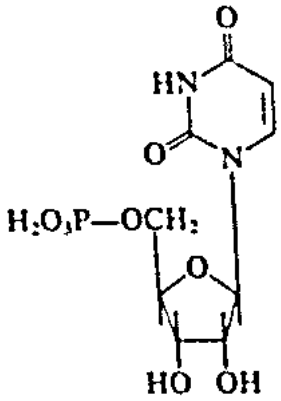
**Uridine**



$(C_9H_{12}O_6N_2)$

MW 244.2 p*K* 9.2 ; 12.5 MP 165 SW +

**Uridine-5'-Monophosphate (UMP)**



$(C_9H_{13}O_9PN_2)$

MW 324.2 p*K* 9.5 MP 200

**Valeric Acid (Pentanoic Acid; Valerianic Acid)**

$H_3C(—CH_2)_3—CO_2H$

$(C_5H_{10}O_2)$

MW 102.1 p*K* 4.8 BP 186 MP −34 SE + + SW 3

**L-Valine (Val)**

$(H_3C)_2—CH—CH(—NH_2)—CO_2H$

$(C_5H_{11}O_2N)$

MW 117.2 p*K* 2.3 ; 9.7 MP 95 SE tr SW 7

**Vitamin $A_1$**

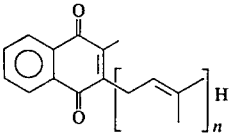
See Retinol.

**Vitamin C**

See L-Ascorbic Acid.

**Table A.16. (*Continued*)**

**Vitamin K**



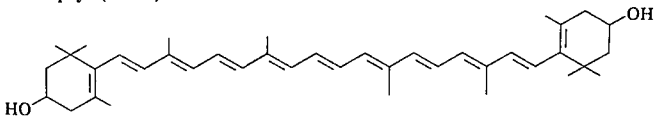
$(C_{11}H_8O_2)$

$n$ = 0 to 9

For $n$ = 0 Menadione = Vitamin $K_3$

MW 172 2 MP 106 SE + SW − —

**Xanthophyll (Lutein)**



$(C_{40}H_{56}O_2)$

MW 568 9 MP 195 SE ++ SW −

**Zeaxanthin**

Isomer of xanthophyll $(C_{40}H_{56}O_2)$

MW 568 9 MP 210 SE + SW −

*Note* BP = boiling point (°C), MP = melting point (°C), MW = molecular weight, p$K$ = p$K_a$ = − log acid, intrinsic dissociation constant[a], SE = solubility in (g/100 ml) ether, SW = solubility in (g/100 ml) water, tr = trace

[a]For amino acids the p$K$s may be of racemic mixtures Underlined values refer to the side chain of the amino acid

## Table A.17. Properties of Lactose

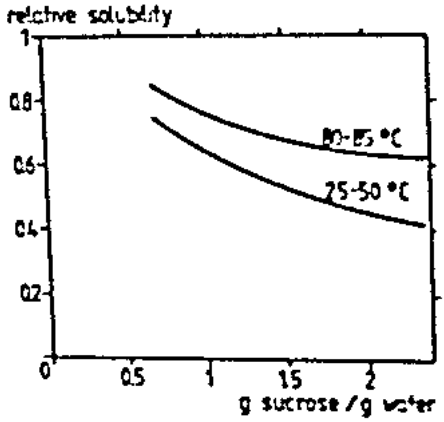
Solubility in water ($q$, in g/100 g water) as a function of temperature ($T$, °C)

$\alpha$-lactose: $\log q = 0.613 + 0.0128\,T$

$\beta$-lactose: $\log q = 1.64 + 0.003\,T$

equilibrium solution: $q = 12.48 + 0.2807T + 5.067 \times 10^{-3}T^2 + 4.168 \times 10^{-6}T^3 + 1.147 \times 10^{-8}T^4$

Effect of sucrose on the solubility, relative to the solubility (g/100 g water) in the absence of sucrose




Diffusion constant of lactose in water at 20°C: $43 \times 10^{-11}$ m²·s⁻¹

| Concentration (g Lactose/ 100 g Water) | Density[a] at 20°C (kg·m⁻³) | Apparent Density[b] (kg·m⁻³) | Viscosity[a] (mPa·s) 20°C | Viscosity[a] (mPa·s) 60°C | Refractive Index[a] 25°C, 589 nm |
|---|---|---|---|---|---|
| 0 | 998.2 | — | 1.00 | 0.47 | 1.3325 |
| 10 | 1043.0 | 1750 | 1.38 | 0.70 | 1.3484 |
| 20 | 1082.0 | 1629 | 2.04 | 0.90 | 1.3659 |
| 30 | 1124.0 | 1592 | 3.42 | 1.29 | |
| 40 | 1173.0 | 1591 | 7.01 | 2.19 | |

Specific rotation [$\alpha$] expressed in degrees of arc per cm path length in a (hypothetical) solution of 1 g lactose in 1 ml, for 5% solutions at 20°C

| Wavelength (nm) | $\alpha$-lactose | $\beta$-lactose | Equilibrium Solutions: Lactose | D-Glucose | D-Galactose |
|---|---|---|---|---|---|
| 589 | +91.1 | +33.5 | + 55.5 | +52.6 | +80.2 |
| 578 | | | + 57.8 | | |
| 546 | +107.2 | +38.7 | + 65.1 | | |
| 436 | | | +105.8 | | |
| 365 | | | +156.0 | | |

**Table A.17. (*Continued*)**

**Properties of lactose crystals**

| Property | α Hydrate | β Anhydrous | α Anhydrous Stable | α Anhydrous Unstable |
|---|---|---|---|---|
| Crystal system | monoclinic | monoclinic | ? | ? |
| Unit cell | | | | |
| *a* axis (nm) | 0.80 | 1.08 | | |
| *b* axis (nm) | 2.17 | 1.33 | | |
| *c* axis (nm) | 0.48 | 0.48 | | |
| β (degrees) | 109.5 | 91.1 | | |
| Density, 20°C ($kg \cdot m^{-3}$) | 1525. | 1589. | 1575. | 1544. |
| Specific heat ($kJ \cdot g^{-1}$) | 1.25 | 1.21 | | |
| Heat of solution[c] ($kJ \cdot mol^{-1}$) | −18.1 | −3.3 | | +10.5 |
| Heat of transition[d] ($kJ \cdot mol^{-1}$) | +1.5 | — | | |
| Melting point[e] (°C) | 201.6 | 252.2 | | 222.8 |
| Solubility, 25°C (g/100 g water) | 8.6 | 51.9 | 35. | 8.6 |

[a]Of solution.
[b]Of lactose dissolved in water.
[c]For a dilute solution.
[d]Transition from α to β
[e]Decomposes.

## Table A.18. Properties of Lactic Acid

**Formula**

$CH_3$–CHOH–$CO_2H$, MW 90.1

**Solubility**

Soluble in water in all proportions, slightly soluble in ethanol and ether (according to pH), not soluble in apolar solvents.

**Isomers**

Both D and L lactic acid occur, specific rotation slight and depending on conditions.

**Melting Point**

D/L lactic acid 16.8°C

**Boiling Point**

D/L lactic acid 122.0°C at 15 mm Hg

**Density**

1250 $kg \cdot m^{-3}$ at 20.0°C

**Esters**

In concentrated solutions (> 6%) biomolecular and higher esters are formed, such as lactoyllactate:

$$CH_3\text{–}CHOH\text{–}CH(\text{–}CH_3)\text{–}CO_2H$$

These esters hydrolyze again after dilution, but slowly; rate of hydrolysis increases with temperature and pH.

## Table A.19. Intrinsic Solubility Products at 20°C

| | | |
|---|---|---|
| $Ca_3Citrate_2$ | $2.3 \times 10^{-18}$ | $mol^5 \cdot kg^{-5}$ |
| $CaHPO_4 \cdot 2H_2O$ | $2.6 \times 10^{-7}$ | $mol^2 \cdot kg^{-2}$ |
| $Ca_4H(PO_4)_3$ | $1.2 \times 10^{-47}$ | $mol^8 \cdot kg^{-8}$ |
| $Ca_3(PO_4)_2$ | $\sim 2.0 \times 10^{-29}$ | $mol^5 \cdot kg^{-5}$ |
| $Ca_5OH(PO_4)_3$ | $\sim 10.0^{-58}$ ? | $mol^9 \cdot kg^{-9}$ |
| $Ca(CH_3CHOHCO_2)_2$ | $\sim 10.0^{-4}$ | $mol^3 \cdot kg^{-3}$ |
| $MgHPO_4 \cdot 3H_2O$ | $1.5 \times 10^{-6}$ | $mol^2 \cdot kg^{-2}$ |

*Note:* From free ion activities in moles per kg water.

**Table A.20. Approximate Intrinsic Association Constants at 20°C (kg water per mole)**

| Anion | Cation | | | | |
|---|---|---|---|---|---|
| | $H^{+a}$ | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ |
| $H_2Citrate^-$ | $1.2 \times 10^3$ | 30 | 15 | 1 | 1 |
| $HCitrate^{2-}$ | $6.0 \times 10^4$ | $10^3$ | 500 | 10 | 10 |
| $Citrate^{3-}$ | $2.5 \times 10^5$ | $10^5$ | $10^5$ | 10 | 10 |
| $H_2PO_4^-$ | 100 | 10 | 10 | 1 | 1 |
| $HPO_4^{2-}$ | $1.8 \times 10^7$ | 600 | 800 | 10 | 10 |
| $PO_4^{3-}$ | $3.0 \times 10^{12}$ | $3.0 \times 10^6$ | $10^5$ | 100 | 100 |
| Glycosyl-$PO_3^{2-}$ | $3.0 \times 10^6$ | 300 | 300 | 10 | 10 |
| $HCO_3^-$ | $2.3 \times 10^6$ | 20 | 20 | 1 | 1 |
| $CO_3^{2-}$ | $2.1 \times 10^{10}$ | $10^3$ | $10^3$ | 10 | 10 |
| $Cl^-$ | < 1 | 10 | 5 | 1 | 1 |
| $SO_4^{2-}$ | 100 | 200 | 200 | 10 | 10 |
| $HC_nH_{2n}CO_2^-$ | $5.0 \times 10^4$ | 10 | 10 | 1 | 1 |
| $CH_3{\cdot}CHOH{\cdot}CO_2^-$ | $8.0 \times 10^3$ | | | | |

[a]Logarithms of values in this column give the intrinsic $pK_a$

## Table A.21. Physical Properties of Milk Fat

| Temp. (°C) | Density ($kg \cdot m^{-3}$) | Refractive Index[a] | Viscosity (mPa·s) | Solubility of Water (% w/w) |
|---|---|---|---|---|
| 10 | 922 | (1.465)[b] | | (0.11) |
| 20 | 915 | 1.462 | 70.8 | (0.14) |
| 30 | 909 | 1.458 | 45.7 | 0.17 |
| 40 | 902 | 1.454 | 30.9 | 0.20 |
| 50 | 895 | 1.451 | 22.1 | 0.24 |
| 60 | 889 | (1.447) | 16.6 | 0.27 |
| 70 | 882 | (1.444) | 12.5 | 0.32 |
| 80 | 876 | (1.440) | 9.8 | 0.36 |
| 90 | (869) | (1.436) | 7.6 | 0.41 |
| 100 | (863) | (1.433) | 6.2 | 0.46 |

Refractive index at 40°C as a function of wavelength ($\lambda$, $\mu$m) is approximately

$$n\lambda - n_D = \left(\frac{5.77\lambda^2 - 0.03}{2.77\lambda^2 - 0.03}\right)^{\frac{1}{2}} - 1.455$$

where $n_D$ = refractive index at $\lambda = 0.589$ $\mu$m.

Heat conductivity: ~0.17 $J \cdot m^{-1} \cdot s^{-1} \cdot K^{-1}$ at room temperature.
Specific heat: ~2.1 $kJ \cdot kg^{-1} \cdot K^{-1}$ at 40°C.
Heat of fusion (total): 85–100 $kJ \cdot kg^{-1}$
Melting dilatation (total): 55–60 $cm^3 \cdot kg^{-1}$
Coefficient of expansion (liquid fat): ~(0.84 + 0.00056 $T$) $cm^3 \cdot kg^{-1} \cdot K^{-1}$, $T$ in °C.
Electrical conductivity: $< 10^{-10}$ $A \cdot V^{-1} \cdot m^{-1}$
Dielectrical constant (relative to that of vacuum): ~3.1, but greatly depending on frequency.
Solubility of air in fat: ~87 $cm^3 \cdot kg^{-1}$, which is made up of 28 $cm^3$ $O_2$ and 57 $cm^3$ $N_2$, all for liquid fat at room temperature in equilibrium with air at atmospheric pressure. Solubility of air in solid fat is negligible.

*Note:* Values are for liquid fat.

[a] At $\lambda = 589$ nm.

[b] Values between brackets are based on extrapolation.

**Table A.22. Density ($\rho$) and Viscosity ($\eta$) of Milk Fractions as a Function of Temperature ($T$)**

| $T$ (°C) | $\rho$ Plasma (kg·m$^{-3}$) | $\rho$ Fat[a] (kg·m$^{-3}$) | $\rho$ Serum[b] (kg·m$^{-3}$) | $\rho$ Water (kg·m$^{-3}$) | $\eta$ Plasma mPa·s | $\eta$ Water mPa·s |
|---|---|---|---|---|---|---|
| 5 | 1035.9 | 959 | 1027.3 | 1000.0 | 2.83 | 1.519 |
| 10 | 1035.2 | 951 | 1027.0 | 999.7 | 2.35 | 1.307 |
| 15 | 1034.4 | 938 | 1026.3 | 999.1 | 1.99 | 1.139 |
| 20 | 1033.3 | 916 | 1025.3 | 998.2 | 1.68 | 1.002 |
| 25 | 1031.9 | 912 | 1024.2 | 997.1 | 1.44 | 0.890 |
| 30 | 1030.0 | 909 | 1022.6 | 995.7 | 1.26 | 0.798 |
| 40 | 1026.1 | 902 | 1019.0 | 992.2 | 1.00 | 0.653 |
| 50 | 1019.8 | 895 | 1014.4 | 988.1 | 0.82 | 0.547 |
| 60 | 1016.6 | 889 | 1009.7 | 983.2 | 0.69 | 0.466 |
| 70 | 1011.2 | 882 | 1004.2 | 977.8 | 0.60 | 0.404 |
| 80 | 1005.0 | 876 | 998.0 | 971.8 | 0.56 | 0.355 |
| 90 | | | | 965.3 | | 0.315 |
| 100 | | | | 958.4 | | 0.282 |

[a]Calculated for $T < 40$°C, taking into account some supercooling.
[b]Calculated.

## Table A.23. Refractive Indices ($n$) of Milk Fractions

| Parameter | Water | Plasma | Serum | Milk Fat |
|---|---|---|---|---|
| $n_D^{20}$ | 1.33295 | 1.34800 | 1.34240 | 1.46200 |
| $dn_D/dT$ ($10^{-4}$ $K^{-1}$)[a] | −1.29 | −1.25 | −1.27 | −3.65 |
| $dn^{40}/d\lambda$ ($10^{-4}$ $nm^{-1}$)[b] | −3.2 | −3.6 | −3.4 | −0.4 |

[a] $T$ = temperature.
[b] $\lambda$ = wavelength; only valid near $\lambda$ = 589 nm (D-line). In many cases $(n^2 + 2)/(n^2 - 1)$ is linear with $\lambda^{-2}$.

## Table A.24. Thermal Properties

| Product | Heat Conductivity ($J \cdot m^{-1} \cdot s^{-1} \cdot K^{-1}$) 20°C | Heat Conductivity ($J \cdot m^{-1} \cdot s^{-1} \cdot K^{-1}$) 80°C | Specific Heat 20°C ($J \cdot g^{-1}$) |
|---|---|---|---|
| Water | 0.59 | 0.67 | 4.14 |
| Serum | 0.54 | 0.64 | (3.95)[c] |
| Plasma | 0.54 | 0.63 | 3.89 |
| Milk | 0.53 | 0.61 | 3.80 |
| Concentrated milk[a] | 0.49 | 0.56 | 3.47 |
| Concentrated milk[b] | 0.46 | 0.53 | 3.23 |
| Cream 20% fat | 0.39 | | 3.55 |
| Cream 40% fat | 0.33 | | 3.26 |
| Milk fat | 0.17 | (0.18)[c] | 2.26 |

*Note.* Approximate values.
[a] Concentrated to 1/1 9 the original mass
[b] Concentrated to 1/2.54 the original mass.
[c] Calculated

## Table A.25. Nutritive Value of Milks

| | | Content per 100 g | | | Daily Requirements[a] | |
|---|---|---|---|---|---|---|
| | | Cow's Milk | | Human Milk | Children[b] | Adults[c] |
| | | Range | Mean | | | |
| Energy | (kJ) | 250–300 | 275 | 300 | 5400 | 11,000 |
| Water | (g) | 85.5–88.7 | 87.3 | 87.1 | | |
| Fat | (g) | 2.4–5.5 | 3.9 | 4.5 | | |
| Protein | (g) | 2.3–4.4 | 3.2 | 0.9 | 23 | 56 |
| Lactose | (g) | 3.8–5.3 | 4.6 | 7.1 | | |
| Ca | (mg) | 60–200 | 125 | 31 | 800 | 800 |
| P total | (mg) | 50–150 | 100 | 15 | 800 | 800 |
| P inorganic | (mg) | | 70 | 12 | | |
| K | (mg) | 100–200 | 150 | 52 | 550–1650 | 1875–5625 |
| Na | (mg) | 20–90 | 44 | 15 | 325–975 | 1100–3300 |
| Cl | (mg) | 60–180 | 105 | 41 | 500–1500 | 1700–5100 |
| Mg | (mg) | 5–24 | 13 | 4 | 150 | 350 |
| Citrate | (mg) | 90–270 | 175 | 40 | | |

[a]U.S. recommended allowance except K, Na, and Cl are "adequate and safe" levels of intake.
[b]1–3 years.
[c]23–50 years, male.

# Index


Accuracy of analysis, 378–380
Acetaldehyde:
  flavor, 345
  formation by heating, 180
Acetic acid, formed by heating, 180
Acetophenone, 345, 351
N-acetylneuraminic acid (NANA), 138
  cleavage from protein, 174
Acidity of milk, 192–197
  changes in, 54
  titratable, 195–196
Acids (organic) in milk, 412–413
Activity:
  of protons, 194
  of salts, 44–47
  of water, 8, 301–311
Adsorption, 212–215
  kinetics of, 214–215
Aflatoxin $M_1$, 141
Age gelation, 166, 246–248
Agglutination of fat globules, 264–265
Agglutinins, 265
Air bubbles, 279–289
Albumin blood serum, *see* Blood serum albumin
Alcohols in milk, 412
Aldehydes:
  *from linoleic acid*, 347
  in milk, 137, 412
Aldolase (fructose-bisphosphate aldolase), 127
Allergens in milk, 367
Amines in milk, 408
Amino acids:
  composition of milk proteins, 402–403
  derivatives in milk, 408–409
  essential, 366
  free in milk, 408
  *in human milk*, 366
  properties of, 100–101
  residues in proteins, 100–101
  sequences in milk proteins, 109, 116, 404–407
p-aminobenzoic acid, 372
Amino sugars in milk, 409
Ammonia, 138
Analysis of milk:
  accuracy, 378–380
  *recalculation*, 380–381
  *sampling*, 377–378
Antibiotics, 143
(–N-β-aspartyl) lysine, 175
Antibodies *see* Immunoglobulins
Arachidonic acid, 364
Ascorbic acid:
  content in milk, 360, 372
  degradation of, 172, 310
  oxidation of, 200
Ash, determination of, 391
Association constants of salts, 453
Atherosclerosis, 365
Autooxidation of linoleic acid, 347
Autooxidation of milk fat, 83–88
  as affected by copper, 85–86
  heat, 166, 172

Autooxidation of milk fat (*Continued*):
processing, 87–88
various conditions, 86–87
water activity, 310
mechanism of, 83–86, 347
products of, in relation to flavor, 343–348

Bacteria:
agglutination in milk, 208
growth as affected by water activity, 310
growth and metabolism, 160
inhibitors of, in milk, 207–209
killing by heat, 172, 209
milk as substrate for, 165, 206–209
Biosynthesis of milk, 12–26
Biotin, 371
Blood serum albumin:
amino acid composition of, 402–403
concentration in milk, 107
properties, 108
structure, 117, 118
Browning, *see* Maillard reaction
Buffalo milk composition, 149
Buffering, 194–196
groups in milk, 194
index, 194
Butter:
composition, 417–418
flavor, 353–354
flavor formulation, 354
volatile compounds in, 353
Buttermilk, composition, 420–421
Buttermilk powder, composition, 420–421
Butter oil, 76–77
Butyric acid, formation by heating, 180

Calcium, quantitation of, 392
Calcium phosphate:
colloidal, 51–53
effect of heat on, 163
Camel milk composition, 149
Carbonates in milk, 43, 49, 50, 136
Carbon dioxide in milk, 43, 49, 50, 136
loss on heating, 163
loss on holding, 53
Carbohydrates of milk, 27–41
other than lactose, 40
Carboxylic acids, 137
Carnitine, 372
Carotenoids, 61
Caseins, 2
amino acid composition, 402–403
association, 113, 114
concentration in milk, 107
interactions, 113, 114
properties, 108
$\alpha s1$-Casein:
amino acid composition, 402–403
amino acid sequence, 109, 405
charge distribution, 109
concentration in milk, 107
genetic variants, 407
properties, 108, 110
$\alpha s2$-Casein:
amino acid composition, 402–403
amino acid sequence, 109, 404
charge distribution, 109
concentration in milk, 107
properties, 108, 110, 111
$\beta$-Casein:
amino acid composition, 402–403
amino acid sequence, 109, 405
charge distribution, 109
concentration in milk, 107
genetic variants, 407
properties, 108, 111
$\gamma$-Casein:
composition, 111–112
concentration in milk, 107
nomenclature, 112
$\kappa$-Casein:
amino acid composition, 402–403
amino acid sequence, 109, 405
charge distribution, 109
concentration in milk, 107
genetic variants, 407
glycosyl groups, 113
properties, 108, 112–113
Casein micelles:
aggregation of, 165, 236, 237
binding by, 237
calcium phosphate in, 51–53
equilibrium with medium, 232
foaming, effect on, 282
"hairy," 230, 231
heating, effect on, 236
model, 231
pH effect on, 236
properties, 5, 6, 229–234
salts effect on, 233
size, 231–233
stability, 234–237
structure, 230
submicelles of, 230
temperature effect on, 234, 236
voluminosity, 231
Casein preparations, 323, 325
Catalase, 127, 129

Cells in milk, 24
Cerebrosides, 61
Cheese, flavor of, 355–357
  volatile compounds in, 356–357
Chloride, quantitation of, 393
Cholecalciferol, *see* Vitamin D, content of milk
Cholesterol, 365
Choline, 372
Churning, 283–285
Chymosin, 238–240
  inactivation by heat, 172
Cisterns, mammary gland, 25
Citrate
  mammary synthesis of, 16, 21
  quantitation of, 393
Clotting:
  by chymosin action, 240–245
  effect of heat treatment on, 166, 245
Clusters of fat globules, 260
Coalescence of fat globules, 261–263
Colloidal phenomena, 218–227
Colloids, hydrophilic, 219–220
Color of milk, 160
  change on heating, 165, 179–181
Colostrum, 9, 152
Components of milk:
  alphabetical list, 423–449
  formulas, 423–449
  principal, 1–3
  properties, 423–449
Composition of dairy products, 160, 416–422
Composition of milk, *see* Milk composition
Concentrating milk:
  by drying, 317–320
  by evaporating, 314–315
  effect on flavor, 350–351
  effect on properties, 307–309
  effect on reaction rates, 310–314
  by freezing, 315–317
  by membrane processes, 320–323
Concentration ratio, 307, 314, 315
Conjugated compounds, 139, 414
Contact angle, 215–217
Contaminants, 9, 140–143
Coprecipitate (proteins), 324
Coronary heart disease, 365
Cottage cheese, composition, 421–422
Cowy flavor, 343
Cream:
  composition, 417–418
  flavor, 352
Creaming, 160, 273–278
  agglutinin, effects of, 277
  heat, effects of, 166
  rate, 273–277
Creatine, 138
Creatinine, 138
Cryoglobulins, 264
Crystallization of milk fat, 92, 160
Cultured products, flavor of, 351–352, 354
  volatile compounds in, 352
Curds, cow *vs* human milk, 367

DDE, 141
Deamidation, 175
Debye-Huckel parameter, 220
Dehydroalanine, 175
Denaturation of proteins, 105–106
  effect of water activity on, 311
  enthalpy of, 169
  entropy of, 169
  free energy change in, 169
  kinetics of, 168
Density:
  of milk, 186–188
  of milk fat, 454–455
  of milk fractions, 455
  of milk products, 187, 416–422
  variation with temperature, 187
Detergents, 143
Diacetyl, flavor, 345
Diameter:
  of fat globules, 255–257, 267
  number average, 227
  volume-surface average, 227
Dielectric constant of milk fat, 454
Diglycerides, 68
Dilatation of milk fat on melting, 454
Dimethyl sulfide, 342, 345
Dimethyl sulfone, 138
Dispersity, changes in, 224–226
Disulfide interchange, 175, 176
DLVO theory (re colloids), 222–223
Donkey milk, composition, 149
Drugs, 143
Dry milk:
  composition, 418–420
  flavor, 350–351
  manufacture, 317–320
  properties, 317–320
Ducts, mammary gland, 25

$E_h$ *see* Redox potential ($E_h$)
Eilers equation (viscosity), 290
Electrical conductivity:
  of milk, 8, 51
  of milk fat, 454
Electrical double layer, 220–221

Electrodialysis, 11
Electrokinetic potential, *see* Zeta potential
Electrostatic repulsion, 222
Emulsifier properties of protein concentrates, 328–330
Emulsion stability, 160
Enzymes, 123–134
  activities in cow's and human milks, 127
  classified list, 409–412
  effect of heat on, 133–134, 164, 172
  effect of water activity on, 310
  flavors caused by, 350
  kinetics of, 123–126
  source and significance, 126
Ergocalciferol, *see* Vitamin D, content of milk
Esterases, 80
Esters in milk, 137, 413
Ethanol in milk, 137
Evaporated milk, 314–315
  composition of, 418–419
Evaporation, 11, 314–315
Expansion, coefficient of (milk fat), 454

Fat globule membrane, 70–76
  composition of, 72–73
  origin of, 71–72
  processing effects on, 75–76, 165–166
  protein in, 121–122
  structure of, 74–75
Fat globules, 3–5
  clustering of, 166, 260
  coalescence of, 166, 261–263, 274
  cold agglutionation of, 264–265, 274
  composition of, 257–258, 274
  floccules of, 260
  in foaming, 283–289
  homogenization of, 266–273
  in recombined milk, 273–274
  size distribution of, 259–257, 274
  stability of, 259–264
  transport in mammary gland, 23
Fat of milk:
  absorption of, 363
  correlation with protein, 148
  crystallization of, 88–96, 160, 258
  digestion of, 363
  "fat constants" of, 401
  fatty acids in, 64–65
  mammary synthesis of, 17–18
  of milk products, 76–78
  neutral lipids in, 399–400
  physical properties of, 454
  rheological properties of, 298–299
  solubility of air in, 454
  solubility of water in, 454
Fatty acids:
  arrangement in triglycerides, 67
  dietary essential, 364
  free in milk, 60, 68–69
  in human milk, 364
  major in milk fat, 64–65
  minor in milk fat, 398–399
  structures of, 59–62
Fermentation, of lactose, 37–40
  of milk, 11
Fermented milks, nutritive value, 375
Flavor:
  analysis, 341–342
  balance, 341–342
  compounds, 338–346
  perception, 336–338
  threshold, 339–340
Flavor, of milk, 160, 352, 350
  of heated milks, 165, 345–349
Flocculation kinetics, 224
Flotation in churning, 284
Foaming, 279–283
  of milk products, 282–283
  of protein preparations, 330–331
Folic acid, 360, 371, 372
Formic acid, production by heating, 180
Fouling, 181–184
Freezing point of milk, 8, 51
Furfural:
  alcohol production by heating, 180
  production by heating, 180, 345, 351

Galactosemia, 361
Galactose in milk, 40
β-galactosidase, 29
Galactosyl transferase, 20, 21, 116, 130
Gangliosides, 61
Gases in milk, 136
Gelation of casein micelles, 225
  by aging, 246–248
  by chymosin, 243–245
Gels, 294–297
  acid vs. rennet, 297
  intermolecular covalent bonding in, 297
  non-covalent bonding in, 297
Ghee, 77
Gibb's equation (adsorption), 212
Glucose, in milk, 40
Glucose phosphate isomerase, 127
(−N-γ-glutamyl) lysine, 175
Glycerides, mono, di, tri, 60
Glycerol ethers, 62–63
Goat milk:
  allergens in, 367
  composition of, 149

gross energy of, 359
Gonadotropin-releasing hormone, 140
Granules of fat, 260
Gross energy of milk, 358, 359, 360

"Half and Half," composition, 416–417
Hamaker constant (re colloids), 222
Heat coagulation, *see* Heat stability of micelles
Heat conductivity of
concentrated milk, 456
cream, 456
milk, 456
milk fat, 454, 456
milk fractions, 456
Heat of fusion of milk fat, 454
Heat stability of micelles, 160, 172, 248–253
effects of additives, 252
composition, 251
concentration, 251–252
homogenization, 253
pH, 249
preheating, 251
temperature, 251
whey proteins, 250
Heat treatment of milk, 10–11
changes produced, 162–167
effects on bacterial inhibitors, 209
flavor, 348–349
lactose, 29
rennet clotting, 245
salts, 55–56
Heavy metals, 143
Hippuric acid, 138
Homogenization, 11, 266–273
clusters formed by, 272
fat globule size, 267–269
new surface layers, 269–270
Hormones, 139, 140
Horse milk composition, 149
Human milk:
composition of, 149
enzymes in, 127
fatty acids in, 364
gross energy of, 359
lactoferrin in, 365, 368
linoleic acid in, 364
lysozyme in, 365, 368
nutrient density of, 359–360
nutritive value of, 457
oligosaccharides in, 362
protein composition of, 365
trace elements in, 396–397
vitamins in, 415
Humidity, relative, *see* Water activity
Hydrogen sulfide
liberated by heat, 176
in milk flavor, 345
5 Hydroxymethylfurfural, production by heating, 180
Hypocholesteremic effect, 365

Ice cream.
composition, 417–418
manufacture, 287–289
Immunoglobulins
in blood, 119
in colostrum, 119
inactivation by heat, 164
in milk, 107, 119
structure of, 118, 119
Indoxyl sulfate, 138
Infant formulas, 374
Infrared absorption.
fat, 384
lactose, 390
protein, 386–387
Inositol, 372
Interesterification, 165
Interfaces, curved, 215
Interfacial tension, 212–214
Ion activity in milk, 44–47
Ionic strength, 8, 221
Ions, transport in mammary gland, 22

Ketones in milk, 137, 412

α-Lactalbumin
amino acid composition, 402–403
amino acid sequence, 116, 406
charge distribution, 116
concentration in milk, 107
genetic variants, 115
heat denaturation, 171
properties, 108
Lactase deficiency, 359, 361
Lactation, milk composition during, 150–152
Lactic acid.
nutritive value, D- *vs* L-, 375
produced by fermentation, 37–38
produced by heating, 180
properties of, 452
Lactoferrin, 120
β-lactoglobulin.
amino acid composition, 402–403
amino acid sequence, 116, 406
charge distribution, 116
concentration in milk, 107
conformational changes, 117
genetic variants 407
heat denaturation, 171

β-lactoglobulin (*Continued*):
properties, 108
structure, 116, 117
Lactones, formation by heating, 165
Lactoperoxidase, 127, 129
Lactose:
chemical properties, 27–30
crystals, 33–37, 451
diffusion constant, 450
effect on absorption of metals, 361, 362
fermentation of, 37–40
heating effects, 29, 163
hydrolysis of, 29
intolerance, 359–361
mammary synthesis, 16, 20–21
modifications, 34
mutarotation, 30–31
nutritive value, 359–360
oxidation, 28
physicochemical properties, 30–37, 450
quantitation, 388–390
solubility, 32, 450
specific rotation, 450
structure, 28
Lactose synthase, 130
Lactulose, 28–30, 172
Lanthionine, 175
Laplace pressure, at interfaces, 215, 217, 218, 280, 281, 282
Lecithin, 60, 69
Light:
absorption of, 190–191
induction of flavors by, 348–349
penetration of, 192
scattering of, 189–190
Linoleic acid:
dietary essentiality, 364
oxidation of, 347
Lipase, lipoprotein, 68, 78–83, 130–131
inactivation by heat, 80, 172
Lipids of milk: 58–97
composition, 58–66
compound, 69–70
mammary synthesis of, 17–18
neutral, 399–400
properties of, 66–70
Lipolysis, 68, 78–83
Lipoic acid, 139, 372, 373
Lipoprotein lipase, *see* Lipase lipoprotein
Lipoprotein particles, 5–6
Lysine loss on heating, 172
Lysinoalanine, 175
Lysozyme, 132
Magnesium, quantitation, 392
Maillard reaction, 165, 172, 177–181, 310–311
Maltol:
flavor, 349
production by heating, 180
Mammary gland structure, 12–14
Marangoni effect, on surfaces, 217
Mastitis effect on composition, 9, 153
Membrane, fat globule, *see* Fat globule membrane
Membrane processes, 11
Methylketones, formation by heating, 165
Methyl sulfide, 138
Micelles, casein, *see* Casein micelles
$\beta_2$-Microglobulin, 120
Microorganisms, flavors caused by, 350
Milk composition, 1–11
age of cow, 153
breed of cow, 149, 150
changes in, 9, 10
changes in ducts and cisterns, 25
correlation of constituents, 157, 158
of individual cows, 150
species differences, 148–149
standardized, 416–417
variability, 148–161
whole, 416–417
Milk concentrates, 301–335
reaction rates in, 310–314
reconstitution and recombination, 331–334
Milk fat, *see* Fat of milk
Milking procedure, changes in composition during, 155
Milk plasma:
definition, 5
physical properties, 455–456
Milk powders, composition, 418–419
Milk products:
composition of, 416–422
lipids in, 77
nutrients in, 373–374
Milk serum:
definition, 6
physical properties, 455–456
Milk structure, 3–8
Mineral nutrients in milk, 360, 362–363
Miscellaneous components of milk, 135–145
Monoglycerides, 68
Mutarotation of lactose, 30–31
Mycotoxins, 143
*myo-inositol*, 372

Proteins of milk (*Continued*):
crosslinking on heating, 175
essential amino acids in, 366
globular, 5
heat induced reactions in, 173–177
mammary biosynthesis of, 18–20
nutritive value of, 365–368
proportions in milk, 106–107
quantitation of, 384–388
transport in mammary gland, 22–23
Protein preparations, 323–327
composition, 324
flavor, 327
functional properties, 326–331
nutritive value, 326–327
solubility, 325, 327
types, 323
Proteose-peptones:
components of, 121
concentration in milk, 107
effect of heating, 174
Pyridoxal, *see* Vitamin $B_6$ milk
Pyridoxamine, *see* $B_6$ in milk
Pyridoxin, *see* Vitamin $B_6$ in milk

Quarg, composition, 421–422

Radionuclides, 143–145
Rancidity:
hydrolytic, 83, 350
oxidative, 85
Recalculation of analyses, 380–381
Recombination of concentrates, 331–334
Recombined fat globules, 273
Reconstitution of concentrates, 331–334
Redox potential ($E_h$), 197–201
effect of heat treatment, 164, 203
formulation, 197, 198
of milk, 199
relation to ascorbate, 199–200
relation to $O_2$ content, 199–200
relation to pH, 198, 199
relation to riboflavin, 200–201
relation to thiols, 201
of various systems, 199
Redox reactions, 201–206
effect of heat treatment, 202
Reducing capacity of milk, 203
effect of heating on, 180
Refractive index, 188–189
contributions of milk constituents to, 188
of milk fat, 454, 456
of milk fractions, 456
Reindeer milk composition, 149
Rennet, *see* Chymosin
Rennetability, 160
Renneting, 237–246
stages in, 237–238
Rennin, *see* Chymosin
Retinol, *see* Vitamin A in milk

Rheological properties, 290–300
of gels, 294–297
of milk fat, 298–299
Newtonian, 290–292
Non-Newtonian, 292–294
Riboflavin in milk, 360, 370
Ribonuclease, 131

Salts of milk, 3, 42–57
association constants of, 453
changes in, 53–56
colloidal composition of, 42–47
dissolved, 47–51
partition, dissolved *vs.* colloidal, 391
quantitation of, 391–393
temperature influence on, 55–56
Sampling for analysis, 377–378
Separation, 11
Serum of milk, 5
Sheep milk composition, 149
Size distributions, 226, 227
of casein micelles, 231–233
of fat globules, 254–257
Skim milk, separated, composition, 416–417
Smoluchovski's equation, re flocculation, 224
Solids, analyses for, 381–382
Solids-not-fat, analyses for, 381–382
Solubility products of salts, 452
Sorption isotherms, 301–303
Specific gravity, 186–188
Specific heat:
concentrated milk, 456
cream, 456
milk, 456
milk fat, 454, 456
milk fractions, 456
Sphingomyelin, 61, 69, 70
Steric repulsion, 223–224
Sterilization, 167
Steroid hormones, 140
Sterols, 61
Stokes parameter of fat globules, 227, 267–268
Stokes velocity, of creaming, 273
Storch and Segelcke rule (for rennet clotting), 243
Streaming, 225, 226
Sulfhydryl oxidase, 127, 129

Sulfur compounds, 138, 139
Superoxide dismutase, 127, 129, 130
Surface area, 227
  of fat globules, 257, 267
Surface chemistry, 211–218
Surface potential, 221
Surface tension, 211, 212
  gradients in, 217–218
Sweetened condensed milk, 315
  composition of, 418–419
Syneresis of rennet clots, 243–245

Taurine, 139, 372, 373
Thermization, 166
Thiamin in milk, 360, 370
Thickening, *see* Age gelation
Thioctic acid, *see* Lipoic acid
Thiocyanate, 139
Thiols (sulfhydryls)
  formation by heat, 164
  oxidation to disulfide, 175, 176
Titratable acidity, effect of heat on, 163
Trace elements, 136
  cow's *vs* human milk, 396–397
  quantitation of, 392
Transferrin, 120
Transport processes, in mammary gland, 21–23
Triglycerides, neutral, 66–68

Ultrafiltration, 11, 321–322
Ultra-high temperature pasteurization, 167
Urea, 138
Uric acid, 138

Van der Waals forces, 218, 222
Variation in milk composition, 8–10, 146–161
  coefficients of, 156, 157
  consequences of, 159–161
  extent of, 155–159
  interspecies in, 147–149
  sources of, 146–155
Viscoelastic behavior, 295
  modulus, 296
Viscosity, 290–294
  heat effects on, 165
  of milk fat, 454–455
  of milk fractions, 455
Vitamin A in milk, 61 360, 368–369
Vitamin $B_1$, *see* Thiamin in milk
Vitamin $B_2$, *see* Riboflavin in milk
Vitamin $B_6$ in milk, 360, 371
Vitamin $B_{12}$ in milk, 360, 372
Vitamin C, *see* Ascorbic acid
Vitamin D content of milk, 360, 369
Vitamin K, 370
Vitamin E in milk, 360, 370
Vitamins in milk:
  cow's *vs* human, 415
  degradation by heat, 165

Water, density, 455
  heat conductivity, 456
  refractive index, 456
  specific heat, 456
  viscosity, 455
Water activity, 8, 301–303
  effect on growth of bacteria, 310–313
    ascorbate degradation, 310
    enzyme activity, 310–313
    lactose crystallization, 312
    lipid oxidation, 310, 311
    Maillard reaction, 310
    protein denaturation, 311
  of milk products, 309
Water binding, 304–307
Whey composition, 416–417
Whey powder, composition, 420–421
Whey proteins
  concentration in milk, 107
  foaming of, 282
  quantitation of, 387–388
  separation of, 114, 115
  solubility of, 163, 172
Whipping, 284–287

Xanthine oxidase, 127, 128

Yak milk composition, 149
Yield of dairy products, dependence on composition, 159
Ymer, composition, 421–422
Yogurt, composition, 420–421
  flavor of, 353
  volatile compounds in, 353
Young's equation (re contact angle), 216

Zebu milk composition, 149
Zeta potential, 221

10174